

MADROÑO

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NOTEWORTHY COLLECTIONS

CALIFORNIA

WAHLENBERGIA MARGINATA (Thunb.) A.DC. (CAMPANULACEAE). —San Francisco Co., San Francisco, Laguna Honda Boulevard, across the street to the west from the Laguna Honda Reservoir, extending approximately 0.25 mile north, south, and east from the intersection of Laguna Honda Boulevard and Clarendon Avenue, in cracks in the sidewalk and road medians, with *Ehrharta erecta* Lam., *Erigeron bonariensis* L., *Oxalis pes-caprae* L., *Polycarpon tetraphyllum* (L.) L. var. *tetraphyllum*, *Pseudognaphalium luteoalbum* (L.) Hilliard & B.L.Burt, *Scrophularia californica* Cham. & Schtdl., *Taraxacum* sp., 37.754299, –122.463844, 120 m, 12 November 2017, *Batiuk 95* (CAS); San Francisco, Lincoln Way, between 34th Avenue and 35th Avenue, in cracks in the central median, 37.764992, –122.493676, 48 m, 12 November 2017, *Batiuk 97* (CAS); San Francisco, at the intersection of Brotherhood Way and Lake Merced Boulevard, in cracks in medians and walkways within the road, 37.714929, –122.485113, 16 m, 12 November 2017, *Batiuk 98* (CAS); western terminus of Freelon Street, in a crack in the gutter, 37.777835, –122.398134, 3 m, 8 June 2019, *Batiuk 165* (CAS); east side of Tunnel Avenue, just north of the San Francisco/San Mateo County line, across from the CalTrain parking lot, in bare soil at the base of a street tree, 37.709097, –122.401286, 8 m, 8 June 2019, *Batiuk 166* (CAS). San Mateo Co., San Bruno Mountain, 0.5 mile west of the county park entrance along the south edge of Guadalupe Canyon Parkway, plants multi-stemmed, branched at base, stems decumbent then erect, corollas light blue to magenta, in a ruderal corridor within coastal scrub on a north-northeast-facing, road-cut in shallow, coarse, rocky, greywacke substrate, with *Avena barbata* Pott ex Link, *Briza maxima* L., *Eschscholzia californica* Cham., *Festuca perennis* (L.) Columbus & J.P.Sm., *Foeniculum vulgare* Mill., *Fragaria chiloensis* (L.) Mill., *Polypodium californicum* Kaulf., *Polystichum munitum* (Kaulf.) C.Presl., *Urospermum picroides* (L.) F.W.Schmidt, et al., 37.69963, 157 m, –122.44283, 31 May 2015, *Schusteff 1* (JEPS); Daly City, John Daly Boulevard, near the intersection with Poncetta Drive and Sheffield Drive, in cracks in the sidewalk and road medians, 37.04404, –122.475573, 48 m, 12 November 2017, *Batiuk 96* (CAS). Sonoma Co., 1 mile east of Glen Ellen, in the northeastern part of Glen Oaks Ranch, perennial herb, plants less than 50 cm tall, densely branched at base, inflorescence branches wand-like, buds pendent, corollas pale to medium blue, in disturbed gravelly clay and wood chip mulch on a south- to southwest-facing slope (flat

to 10% slope in the immediate vicinity), tree or shrub canopy absent, with *Adenostoma fasciculatum* Hook. & Arn., *Hypericum concinnum* Benth., *Hypochaeris glabra* L., *Plantago erecta* E.Morris, *P. lanceolata* L., *Sanicula laciniata* Hook. & Arn., et al., 38.37247, –122.50198, 168 m, 27 March 2013, *Warner 271* (CDA, SD, UCR); northeastern part of Glen Oaks Ranch, along lower 0.25 mile of chaparral trail, disturbed gravelly clay and wood-chip mulch, 38.375946, –122.499476, 144 m, 3 April 2013, *Warner 279* (CDA); southern Mayacamas Mountains, southeast of Cavedale Road at #4000, north of the junction with Nicolini Road in the upper east fork of the Hooker Creek watershed, in knobcone pine woodland - mixed chaparral (burned in October 2017), 15% slope with a 180° aspect, on Sonoma Volcanics (andesite, basalt, rhyolite), with *Aira caryophyllea* L., *Acmispon brachycarpus* (Benth.) D.D.Sokoloff, *Adenostoma fasciculatum*, *Arctostaphylos glandulosa* Eastw., *Castilleja foliolosa* Hook. & Arn., *Chlorogalum pomeridianum* (DC.) Kunth., *Crocanthemum scoparium* (Nutt.) Millsp., *Eriodictyon californicum* (Hook. & Arn.) Torr., *Festuca bromoides* L., *Stipa lepida* Hitchc., and *Toxicoscordion fremontii* (Torr.) Rydb., 544 m, 12 June 2018, *Warner 437A, 437B* (CAS); Glen Ellen, northeast portion of Glen Oaks Ranch, in roadbed in rocky, volcanic chaparral that burned in 2017, 38.375946, –122.499476, 226 m, 30 April 2019, *Batiuk 150 with A. Schusteff and P. Warner* (CAS).

Previous knowledge. *Wahlenbergia marginata* is a taprooted perennial herb that in recent treatments is considered native either to southeast Asia broadly (Lammers 2007; Hong and Lammers 2011) or to Japan only (Pettersen 1997a, b, 2005). It has been recorded throughout southeast Asia, where it ranges from India and Nepal east to Vietnam, southern China, the Philippines, Korea, and Japan (Lammers 2007), as well as Oceania, Hawaii, and southeastern South America (GBIF 2019). However, Smith (1992) and Cupido et al. (2013) no longer recognize it as occurring in mainland Australia, and Pettersen (1997a, b, 2005) no longer recognizes it as occurring in New Zealand. In North America, where an accepted common name is “southern rockbell,” *W. marginata* is naturalized in the southeastern U.S., from Texas east to North Carolina (Kartesz 2015; Wunderlin et al. 2019; USDA-NRCS 2019). It was first documented in the U.S. in 1937 (Ward 1978).

Wahlenbergia marginata is part of a complex that is “notoriously difficult taxonomically and nomenclaturally” (N. Morin, The University and Jepson Herbaria, personal communication). The authors have had specimens verified as *W. marginata* by Dr. Tom Daniel at the California Academy of Sciences

(Batiuk 95, 97, 98 [CAS]), though Dr. Nancy Morin states that the complex “is probably in need of a full revision” (N. Morin, The University and Jepson Herbaria, personal communication). Corolla morphology, an important identifying character in the genus *Wahlenbergia*, is variable within plants called *W. marginata*. To use the U.S. as an example, California plants agree with Petterson’s (1997a, 2005) descriptions and diagrams of *W. marginata*, which are based on the type locality in Honshu, Japan. In particular, the corollas are nearly rotate, and the calyx lobes consistently exceed the corolla tube. However, the authors have examined herbarium specimens (at the California Academy of Sciences in San Francisco) and digital scans of herbarium specimens (Wunderlin et al. 2019, New York Botanic Garden 2020) of plants called *W. marginata* from several states in the southeastern U.S., and they have infundibular corollas, with the tube exceeding the calyx lobes. In correspondence with Nancy Morin, Mats Thulin, an expert in African *Wahlenbergia*, stated that variation in *W. marginata* might encompass both the California plants and the southeastern U.S. plants, but he suggested that they might have originated from different sources (N. Morin, The University and Jepson Herbaria, personal communication).

In California, a plant identified as *Wahlenbergia gracilis* (G.Forst) A.DC. was collected on San Bruno Mountain in San Mateo County in 1976 (Hewlett s.n., JEPS). *W. gracilis* has been treated as a synonym, at least in part, under *W. marginata* in some references (Petterson 1997a, Hong and Lammers 2011, Tropicos.org 2019, Wunderlin et al. 2019), though Petterson (1997a) also treats these taxa as distinct under a narrow circumscription, as does Smith (1992). The authors could not relocate the precise location of the Hewlett collection, but they have observed and collected *W. marginata* along Guadalupe Canyon Parkway in the near vicinity of the Hewlett collection. The Hewlett specimen compares well vegetatively and in capsule morphology (obconic) to *W. marginata* collected and observed by the authors, but the corollas on Hewlett’s voucher were in poor condition, and therefore, the authors could not make a conclusive comparison.

Jake Sigg states that he knew *Wahlenbergia marginata* from San Francisco since sometime in the mid- to late 2000s (J. Sigg, California Native Plant Society, Yerba Buena Chapter, personal communication), but he did not document it with a voucher specimen, and he did not report it until 2019. It had been known from Sonoma County at Sugarloaf Ridge State Park since 2011, but it was not reported until 2015, via iNaturalist (Kevin G. Hints, personal communication; iNaturalist 2019). At present, *W. marginata* has not been included in any of the existing published treatments for the California flora known to the authors, nor is it included in Hrusa et al. (2002) or Dean et al. (2008).

Significance. These are the first collections of *W. marginata* recorded for San Francisco and Sonoma counties. This is the first collection for San Mateo County since at least 1976, depending on the ultimate determination of the 1976 Hewlett collection. Beyond these collections, the authors have observed *W. marginata* to be widespread in urban San Francisco and locally abundant in Sonoma County at Glen Oaks Ranch and along a short length of the Lower Bald Mountain trail at Sugarloaf Ridge State Park. While the San Francisco County and San Mateo County plants are typically weeds of disturbed areas such as roads, sidewalks, and trails, at Glen Oaks Ranch, plants have been observed 100 or more meters away from any road or trail in dense chaparral and knobcone pine forest that burned in the 2017 Tubbs Fire. Although observations to date do not suggest that this species is a strong invader capable of substantially displacing or disrupting elements of California’s wildlands, it is clearly established and naturalized (sensu Jepson Flora Project 2019) in wildland ecosystems outside of disturbance corridors and has potential to continue spreading into other such areas.

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NOTEWORTHY COLLECTIONS

IDAHO

Platanthera ephemerantha R.M.Bateman (ORCHIDACEAE). —Clearwater County, Elk Creek Falls trail, east-facing slope of Tick Ridge in mixed conifer forest, vicinity of 46.735930°, -116.180220°, elev. 770–790 m, 15 July 2018, *Riser 1062*, *Riser 1063*, and *Riser 1064* (WS). Occasional individuals and small groups growing in deep conifer litter alongside and adjacent to trail just above lower Elk Creek Falls. Associated species include: *Abies grandis* (Douglas ex D.Don) Lindl., *Actaea rubra* (Aiton) Willd., *Cephalanthera austini* (A.Gray) A.Heller, *Coptis occidentalis* (Nutt.) Torr. & A.Gray, *Coralorrhiza maculata* (Raf.) Raf., *Goodyera oblongifolia* Raf., *Pinus monticola* D.Don, *Platanthera elegans* Lindl., *Pseudotsuga menziesii* (Mirb.) Franco, *Thuja plicata* Donn ex D.Don, and *Vaccinium membranaceum* Douglas ex Torr.

Previous knowledge. *Platanthera ephemerantha* (syn. *Piperia candida* Rand. Morgan & Ackerman; Bateman et al. 2009) is generally regarded as having a coastal distribution “not more than 150 km from coast” (Ackerman and Morgan 2002) stretching from southern Alaska through coastal British Columbia, Washington, and Oregon, south to central coastal California just north of Monterey Bay (Morgan and Ackerman 1990; Ackerman and Morgan 2002; Ackerman and Lauri 2012; Riser 2015; Legler 2018). In Oregon and Washington, this infrequently collected species is known from a few collections occurring slightly east of the Cascade crest (Consortium of Pacific Northwest Herbaria 2010+). In 2009, Curtis Björk collected *P. ephemerantha* (as *Piperia candida*, *Björk 18894*) from east of Dworshak Reservoir, Clearwater County, in Idaho. In his study of coastal disjunct species in the interior wetbelt region of the Rocky Mountains, he mentioned this occurrence as being one of four unreported coastal disjunct taxa from this region (Björk 2010).

Significance. Despite the previous collection by Björk, botanists and orchid researchers have overlooked the occurrence of this species in Idaho. In addition to the collections presented here, Mike Hays, Forest Botanist for the Nez Perce-Clearwater National Forest, has observed *Platanthera ephemerantha* along the slopes above Lower Twin Creek in the North Fork of the Clearwater drainage, Idaho (M. Hays, U.S. Forest Service, personal communication). These collections and observation document a disjunction of over 420 km east of the currently recognized distribution for *P. ephemerantha*. This interior wetbelt region and its coastal disjunct species has been the subject of several studies (see references in Björk 2010) and thus it is surprising this species

has been overlooked for so long. Likely, this is due to it being mistakenly collected as *P. unalascensis* (Spreng.) Kurtz. Living plants of *P. ephemerantha* are readily distinguished from *P. unalascensis*: white versus greenish flowers with mostly secund versus spiral flower presentation, respectively (see Morgan and Ackerman 1990 for further identification details). However, pressed specimens can be exceedingly difficult (or impossible) to reliably assign to species without notes regarding flower color and/or orientation, which are nearly always lacking, especially in earlier collections. Likely some *P. unalascensis* collections from this region actually represent *P. ephemerantha*. This highlights the need for collectors to record detailed information regarding flower color and orientation if these key features will be lost or obscured upon pressing.

—JAMES P. RISER II, jriserii@gmail.com.

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LEAF HAIR TUFTS FUNCTION AS DOMATIA FOR MITES IN *QUERCUS AGRIFOLIA* (FAGACEAE)

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ABSTRACT

An identifying feature of *Quercus agrifolia* Née (Fagaceae) is the presence of hair tufts on lower leaf surfaces. In other plant species, hair tufts act as domatia for arthropods such as mites, which in turn feed on leaf fungi or small herbivores and possibly benefit plant health. However, this mutualistic relationship remains untested in *Q. agrifolia*. In this study two primary questions were addressed within a natural stand of *Q. agrifolia* in San Luis Obispo, CA: 1) Do hair tufts act as domatia for mites? and 2) Does the removal of hair tufts impact mite abundance, herbivory or fungal pathogens on leaves? In an observational study of 377 leaves from 20 trees, we found a significant association between the presence of hair tufts and the presence of mites. When we experimentally removed hair tufts, we found a significant reduction in mites, yet there was no impact on leaf herbivory or necrosis. We conclude leaf hair tufts on *Q. agrifolia* serve as domatia for mites, but we found no evidence that mites reduce herbivory or fungal pathogens. Thus, while mites likely benefit from housing provided by hair tufts on *Q. agrifolia*, it is unclear that the tree benefits from the mites, i.e., whether this is a mutually beneficial relationship.

Key Words: acrodomatia, coast live oak, commensalism, mite, mutualism, plant-animal interaction.

Leaf domatia are protective structures or ‘houses’ for arthropods and may represent a plant-arthropod mutualism. The arthropod benefits from protection provided by the host plant (e.g., Norton et al. 2001; Faraji et al. 2002; Grostal and O’Dowd 1994), while the host plant benefits from the arthropods’ active removal of herbivores or fungal pathogens (e.g., Agrawal and Karban 1997; Romero and Benson 2004; Norton et al. 2000; Weber et al. 2016). Across angiosperms, domatia have evolved independently hundreds of times and display a wide range of morphologies including tiny caves, pits, or hair tufts (O’Dowd and Willson 1989).

On the central coast of California, hair tufts on the abaxial leaf surface of *Quercus agrifolia* Née (coast live oak) are commonly observed (Fig. 1) and are a key feature used to distinguish this species from other California live oaks (Baldwin 2012). Previous work in the northern portion of the range of *Q. agrifolia* found that predatory and fungivorous mites (*Tydeus* sp.) occupied 34% of leaf hair tufts (Pemberton and

Turner 1989). However, it is unclear whether mites were more likely to occupy hair tufts than other areas of the leaf (i.e., whether hair tufts function as domatia) and, if so, whether mites benefit tree health by reducing leaf herbivores or fungal pathogens.

In this study we address three specific questions within a southern population of *Q. agrifolia*: Is there variation in the number of leaf hair tufts among individual trees? Are mites more likely to be found in hair tufts as opposed to other areas of the leaf? And finally, does the removal of hair tufts impact the number of mites, herbivory, and fungal damage?

METHODS

Study Design

This study took place in a natural stand of *Quercus agrifolia* in “Poly Canyon” at California Polytechnic State University, San Luis Obispo, California (latitude 35.3057, longitude –120.6581).

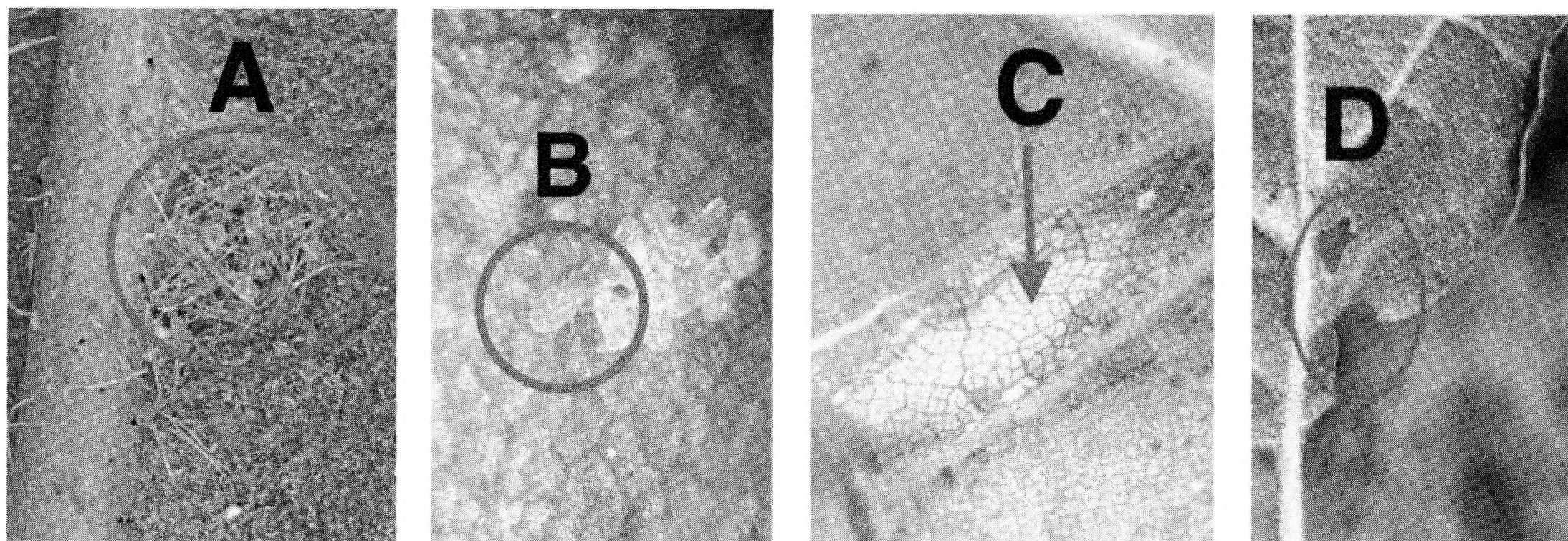


FIG. 1. *Quercus agrifolia* abaxial hair tufts (A), predatory mite in the family Phytoseiidae (B), necrosis due to fungal or bacterial pathogens (C), and herbivory (D)

To address our first study question, we conducted an observational study on 22 February 2019. Twenty teams, each comprised of two to four students, were assigned a random GPS coordinate within the study population and sampled leaves from the nearest tree. Random GPS coordinates were obtained by creating a roughly nine-acre polygon around oak woodland habitat in Poly Canyon using ArcGIS software (ArcGIS release 10.6.1, ESRI, Redlands, CA) and randomly sampling coordinates from within the polygon. Thirty leaves were haphazardly sampled, each from a unique branch within the sub-canopy. For each leaf, the petiole was cut using a razor blade to avoid flinging any mites off the leaf and leaves were placed individually in a plastic bag. Bags were carefully transported back to the lab in plastic trays so as to cause minimal disturbance to any mites.

In the lab, each team of students recorded data on 2–3 leaves from each of the 20 sample trees. This was done in order to avoid confounding the effects of student group and sample tree. For each leaf, the number of leaf vein axils with hair tufts was recorded. Hair tufts were defined as clusters of three or more hairs. For example, in Fig. 2, leaf secondary vein axils 3 and 6 contain hair tufts. To determine the association between hair tufts and mites, a single axil on each leaf was observed for the presence or absence of mites. All observations were made with a dissecting microscope at 10–40X magnification. Mite presence included larvae, nymph, or adult mites (eggs were excluded due to uncertainty in their identification). In order to sample approximately the same number of ‘no hair tuft’ as ‘hair tuft’ leaf axils for each tree, each leaf was pre-assigned to either the ‘no hair tuft’ or ‘hair tuft’ category. If ‘no hair tuft’ category was assigned, an axil without a hair tuft was selected for observation, always beginning with the second leaf vein axil from the bottom and to the right of the mid vein (Fig. 2, axil 2), and moving counterclockwise until an axil without hair tufts was located (in Fig. 2 this would be axil 2). Upon locating a suitable axil, the absence or presence of

mites was recorded. The same protocol was used for sampling leaves pre-assigned to the ‘hair tuft’ category (in Fig. 2 this would be axil 3).

Next, we performed a manipulative experiment to determine whether removal of hair tufts impacted

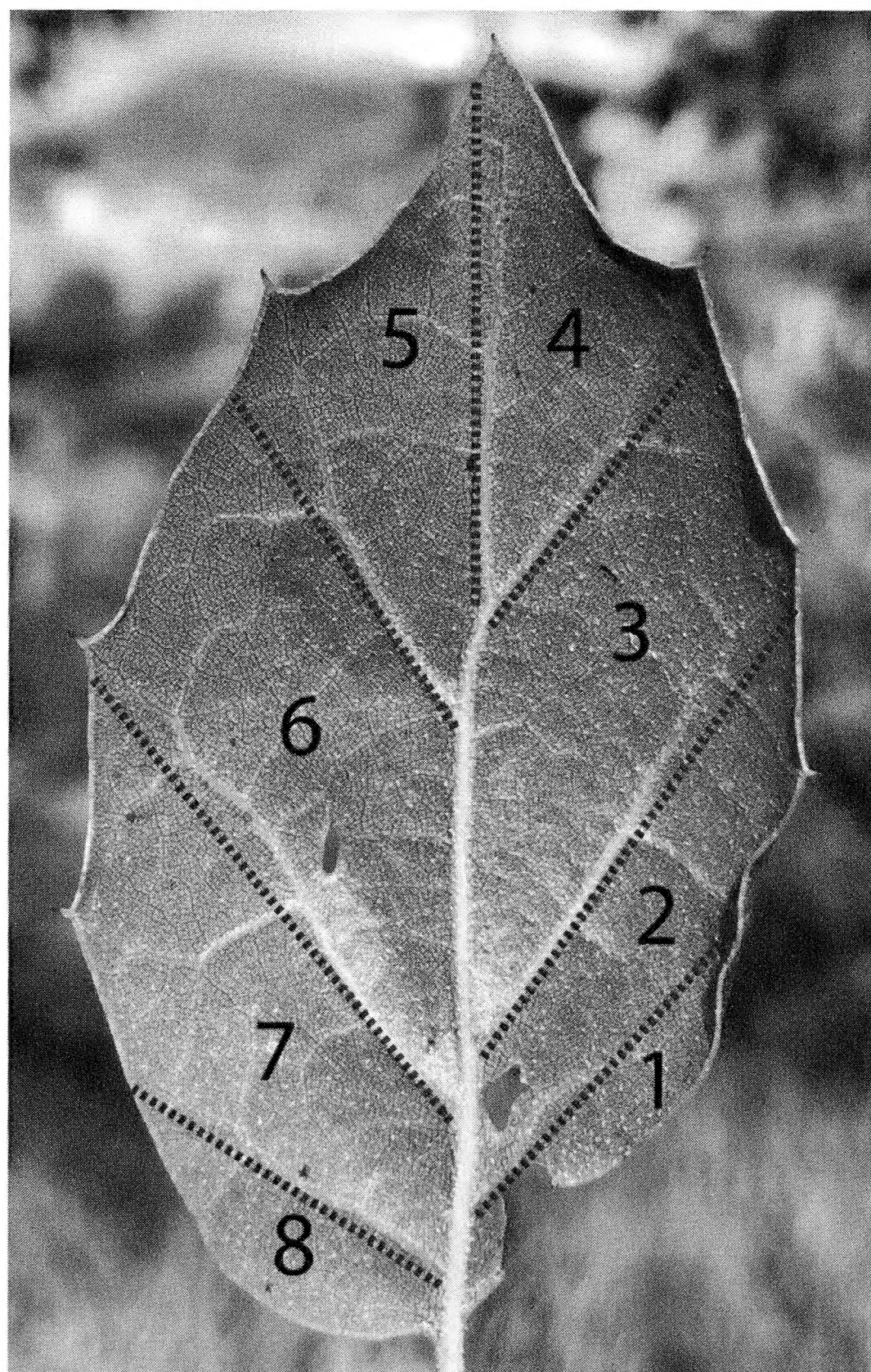


FIG. 2. Method of partitioning leaf surface for estimating necrosis and herbivory based on secondary leaf veins. Note hair tufts are present in vein axils 3 and 6.

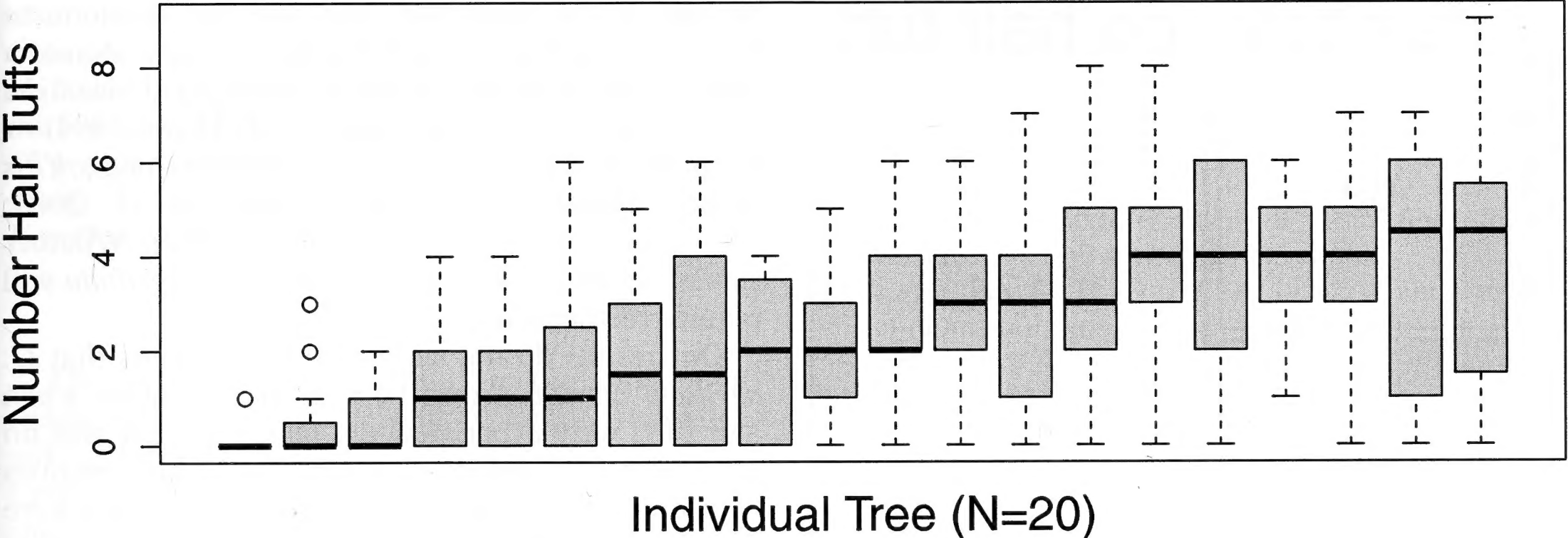


FIG. 3. Boxplot indicating variation in the number of hair tufts per leaf across *Quercus agrifolia* trees, ordered by median value. Number of leaves sampled per tree ranged from 18–24, for a total of 397 leaves.

the abundance of mites and leaf health. This experiment took place between 25 February through 10 May 2019, using a paired study design on 20 haphazardly sampled trees. Within each tree, 10 branches were selected that met the following criteria: branches contained at least 5 fully expanded leaves, were close enough to the ground to allow easy access by researchers, and were not in contact with any neighboring branches (to reduce the opportunity for mites to move from untreated to treated branches). Branches were then randomly assigned as removal or control treatment. On the ‘removal treatment’ branches, all hair tufts located in leaf vein axils on the abaxial surface were removed by gently scraping the leaf surface with a sharp tool (e.g. a scalpel). This was performed on every leaf within the branch. On ‘control treatment’ branches, abaxial leaf surfaces were scraped immediately adjacent to but just outside all leaf vein axils with hair tufts, thus leaving the hair tufts intact. The control treatment accounts for any effect of leaf scraping, for example, potential damage to the epidermis of the leaf.

Treatments were left untouched for 4–6 weeks, at which point we returned to assess mite abundance and leaf health. On each branch, we removed three leaves, always sampling the 3rd, 4th, and 5th fully expanded leaf from the apical meristem. The three leaves were placed in a plastic bag, transported back to the laboratory, and assessed within 24 hours of collection. Using a dissecting scope, on each leaf we recorded the number of leaf vein axils with: hair tufts, mites, herbivory, necrosis due to fungal or bacterial pathogens, and fly larvae/larvae scars (Fig. 1). Herbivory was defined as leaf areas with visible physical damage, while necrosis was defined as leaf areas with visible discoloration or decay. Fly larvae/larvae scars were not included in downstream analyses, as only 27 of 450 leaves had any presence of larvae/larvae scars.

Statistical Analyses

To assess whether there was phenotypic variation among trees in the number of hair tufts per leaf, we used a generalized linear model with a Poisson error distribution (base stats package in R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The response variable was ‘number of hair tufts’ while the predictor variable was tree identity ($n = 20$).

To determine whether there was an association between hair tufts and mites, we used a mixed effects logistic regression (*glmer* function in the *lme4* R package, Bates et al. 2015). Abaxial leaf vein type was included as a fixed effect (hair tufts vs. no hair tufts), while tree identity ($n = 20$) and observer group ($n = 20$) were included as random effects. The binary response variable was mite presence vs. absence.

To assess whether there was an effect of experimental treatment (control vs. hair tuft removal) on mite abundance and leaf health we used a mixed effects linear regression with a Poisson error distribution (*glmer* function in the *lme4* R package, Bates et al. 2015). Treatment was included as a fixed effect (control vs. hair tuft removal), while branch nested within tree identity ($n = 20$) was included as a random effect. Three separate models were created for the following response variables: count of abaxial leaf veins with mites, herbivory, and necrosis. Code for all analyses and raw data files are available on the Dryad Digital Repository (<https://doi.org/10.5061/dryad.4f4qrfjb4>).

RESULTS

Trees exhibited significant variation in the average number of abaxial leaf vein axils with hair tufts: average $2.4 \pm 1.3SD$ hair tufts across 20 trees (Fig. 3; generalized linear mixed model, $F\text{-value} = 10.29$, $df = 19$, $P < 0.001$). Overall, there was a significant association between mites and abaxial hair tufts—the odds of mites being present were 9.4 times greater

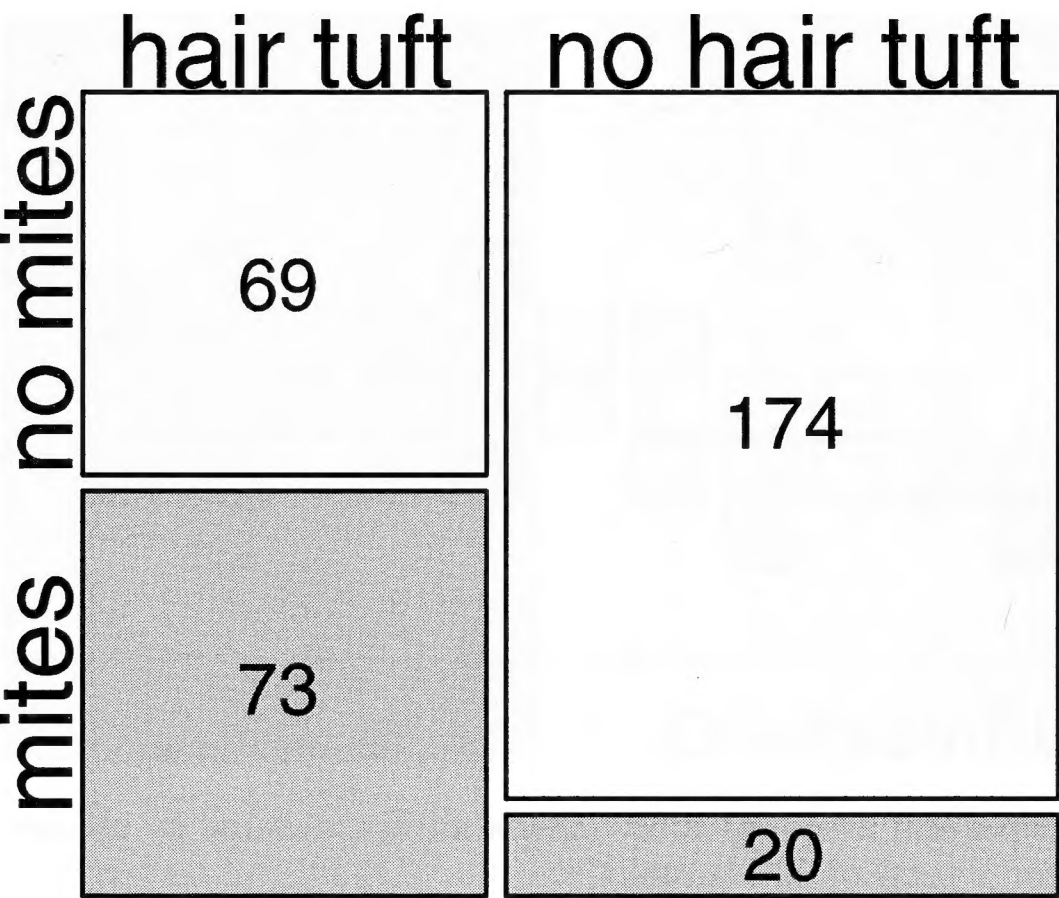


FIG. 4. The distribution of mites in leaf vein axils with and without hair tufts. Numbers indicate the total leaf count within each category.

when abaxial hair tufts were present (Fig. 4; mixed effects logistic regression, estimated log odds ratio 9.4 with 95% CI of 5.2–16.7, $P < 0.001$).

After experimentally removing hair tufts, the number of leaf vein axils containing mites was reduced by 31% relative to the control treatment (Fig. 5; generalized linear mixed model, $\chi^2 = 6.96$, $df = 1$, $P = 0.008$). There was no significant difference in the number of leaf axils with herbivory or necrosis in the control versus removal treatment (Fig. 5, generalized linear mixed model, $\chi^2 = 0.04$, 0.03 , $df = 1,1$, $P = 0.828$, 0.865 for herbivory and necrosis respectively).

DISCUSSION

These results support the hypothesis that leaf hair tufts on *Quercus agrifolia* function as domatia for mites: mites were more likely to occupy leaf vein axils with than without hair tufts, and the removal of hair tufts led to a reduction in mite abundance. We conclude that leaf hair tufts on *Q. agrifolia* likely

benefit mites, however, the specific mechanism remains untested. In other plant species, domatia were found to benefit mites by reducing desiccation (e.g., *Viburnum tinus*, Grostal and O’Dowd 1994) or by providing protection from predators (e.g., *Vitis riparia* (Michx.) A.Gray, Norton et al. 2001; *Capsicum annuum* L., Faraji et al. 2002). Whether similar mechanisms are occurring in *Q. agrifolia* will require further study.

Our results do not support the hypothesis that *Q. agrifolia* benefits from the presence of mites: when hair tufts were experimentally removed there was no impact on leaf herbivory or necrosis. This contrasts with other plant species where predatory mites were found to reduce damage from pests (e.g., *Gossypium hirsutum* L., Argawal and Karban 1997; *Cupania vernalis* Cambess., Romero and Benson 2004) and fungivorous mites were found to reduce powdery mildew (e.g., *Vitis riparia* A.Gray, Norton et al. 2000; Weber et al. 2016). In the present study, two commonly observed mites were identified to family: Phytoseiidae and Tydeidae. Family Phytoseiidae contains predatory mites that are efficient predators of spider mites, gall mites, and other plant pests (David Walter, University of the Sunshine Coast, Queensland, Australia, personal communication). Family Tydeidae contains both fungivorous and predatory mites.

While this system may indeed represent a commensalism where mites benefit from *Q. agrifolia*, but *Q. agrifolia* does not benefit from mites, we cannot yet rule out a mutualistic relationship. First, our experimental treatments only reduced mite daytime abundance by 36%. Because mites are active at night and are known to move between branches (Weber, personal observation), it could be that our experimental branches (containing ca. 5-15 leaves) were not large enough to restrict movement of mites during the night from adjacent untreated branches. Secondly, the benefits provided by mites may only occur during a specific time of year or under specific environmental conditions not captured during our ca. 10-week study interval in spring of 2019. For

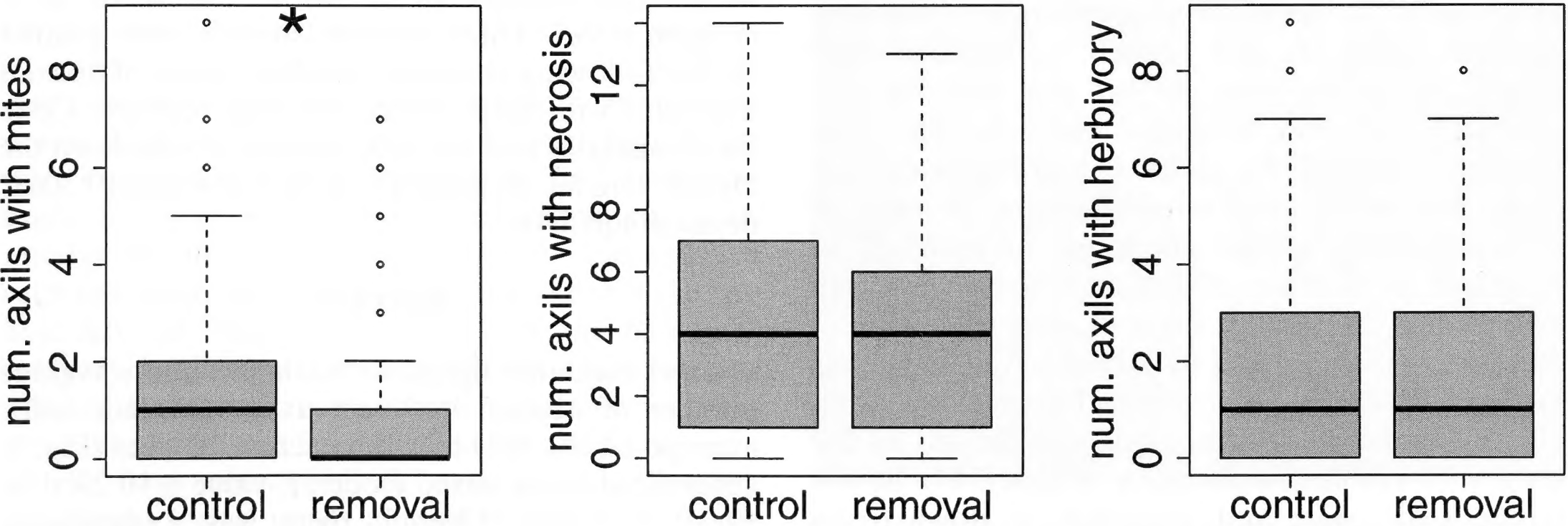


FIG. 5. Number of leaf vein axils with mites, necrosis (due to fungal or bacterial pathogens), and herbivory in control vs. hair tuft removal treatments. An asterisk (*) signifies $P < 0.05$.

example, in rare outbreak conditions of a pathogen, mite domatia may impact fungal damage (Norton et al. 2000), even if there is no detectable impact in non-outbreak years. We recently confirmed the presence of powdery mildew on an individual of *Q. agrifolia* about 10 miles west of our study site in the town of Los Osos, CA.

Evolutionary Implications

The evolution of domatia in the oak family (Fagaceae) remains largely unexplored. At least 25 oak species from subsections Lobatea, *Quercus*, *Cerris* and *Ilex* are known to have hair tufts, five species from subsections *Quercus* and *Cyclobalanopsis* lack hair tufts, while two species are putatively polymorphic: *Q. robur* and *Q. serrata* (Brouwer and Clifford 1990; O'Dowd and Pemberton 1994, 1998; O'Dowd and Willson 1997). Since no systematic review of domatia across oaks has yet been made, it is unclear whether domatia have evolved once or multiple times across this family.

Across all angiosperms, domatia likely evolved hundreds of times (O'Dowd and Willson 1989), yet few studies have directly examined natural selection and evolution of this key trait involved in mediating plant-arthropod interactions (but see Agrawal and Karban 1997). Within our study population, phenotypic variation in hair tuft number was present across trees. If this variation is heritable, it would provide the raw fuel for evolution and merit further study. While estimating selection in such a long-lived organism may seem unfeasible, oak tree seedlings and juvenile plants often exhibit hair tufts (Grossenbacher, personal observation). Thus, it may be feasible to estimate heritability and observe selection (at least during early life history stages) in *Q. agrifolia*, making it a system worth considering for further study.

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TAXONOMIC REVISION OF *MENTZELIA* SECTION *BICUSPIDARIA* (LOASACEAE) IN MEXICO

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ABSTRACT

Results from molecular phylogenetic analyses have indicated that the two southernmost species in *Mentzelia* section *Bicuspidaria*, *M. hirsutissima* sensu lato and *M. involucrata*, form a clade in which *M. hirsutissima* is paraphyletic with respect to *M. involucrata*. Here we elevate *M. hirsutissima* S.Watson var. *nesiotes* I.M.Johnst. to specific rank, recognizing it as *M. nesiotes* (I.M.Johnst.) Brokaw & J.J.Schenk, thus rendering all three taxa monophyletic. *Mentzelia nesiotes* is allopatric relative to *M. involucrata* and *M. hirsutissima* sensu stricto, with all known populations of *M. nesiotes* occurring in northern Baja California Sur and nearby Pacific islands. Multivariate analyses of morphological differences determined that *M. nesiotes* and *M. hirsutissima* are best distinguished by capsule length, calyx length, and leaf intersinus width. Estimation of climate niches with MaxEnt inferred that *M. nesiotes* and *M. hirsutissima* showed minimal overlap in climate tolerances, with *M. nesiotes* inhabiting the Vizcaíno fog desert west of the Peninsular Ranges and *M. hirsutissima* inhabiting areas further north with higher precipitation and temperature fluctuations primarily east of the Peninsular Ranges. We provide updated circumscriptions of all three species, reduce *M. hirsutissima* var. *stenophylla* and *M. involucrata* var. *megalantha* to synonymy with their respective species, and designate the lectotype of *M. hirsutissima*.

RESUMEN

Los resultados de los análisis filogenéticos moleculares han indicado que las dos especies más al sur de *Mentzelia* sección *Bicuspidaria*, *M. hirsutissima* sensu lato y *M. involucrata*, forman un clado en el que *M. hirsutissima* es parafilética con respecto a *M. involucrata*. Aquí elevamos *M. hirsutissima* S.Watson variedad *nesiotes* I.M.Johnst. a un rango específico, reconociéndolo como *M. nesiotes* (I.M.Johnst.) Brokaw & J.J.Schenk, haciendo que los tres taxones sean monofiléticos. *Mentzelia nesiotes* es alopátrica en relación con *M. involucrata* y *M. hirsutissima* sensu stricto, con todas las poblaciones conocidas de *M. nesiotes* que ocurren en el norte de Baja California Sur y las islas del Pacífico cercanas. Los análisis multivariados de las diferencias morfológicas determinaron que *M. nesiotes* y *M. hirsutissima* se distinguen mejor por la longitud de la cápsula, la longitud del cáliz y el ancho de la hoja. La estimación de nichos climáticos con MaxEnt infirió que *M. nesiotes* y *M. hirsutissima* mostraron una superposición mínima en las tolerancias climáticas, con *M. nesiotes* que habitaban en el desierto de niebla de Vizcaíno al oeste de los Rangos Peninsulares y *M. hirsutissima* habitando áreas más al norte con mayores precipitaciones y fluctuaciones de temperatura principalmente al este de las Cordilleras Peninsulares. Proveemos circunscripciones actualizadas de las tres especies, reducimos *M. hirsutissima* variedad *stenophylla* y *M. involucrata* variedad *megalantha* para que sean sinónimos con sus respectivas especies, y designar el lectotipo de *M. hirsutissima*.

Key Words: Baja California Sur, climate niche, *Mentzelia hirsutissima*, *Mentzelia involucrata*, *Mentzelia nesiotes*, *Mentzelia* section *Bicuspidaria*, species distribution models, Vizcaíno Desert.

A recent molecular phylogenetic study of *Mentzelia* L. section *Bicuspidaria* S.Watson (Brokaw et al. 2020) determined that species of section *Bicuspidaria* that were at least partially distributed in Mexico, including *M. hirsutissima* S.Watson and *M. involucrata* S.Watson, form a monophyletic group sister to a clade of the remaining species that occur only in the United States, including *M. reflexa* Coville, *M. tricuspis* A.Gray, and *M. tridentata* (Davidson) H.J.Thomps. & J.E.Roberts (Fig. 1). However, populations of *M. hirsutissima* sensu lato were paraphyletic with respect to *M. involucrata* (Fig. 1).

Northern populations of *M. hirsutissima* from northern Baja California and southern California formed a clade that was sister to *M. involucrata*, whereas southern populations of *M. hirsutissima* from northern Baja California Sur and adjacent Pacific islands formed a more distantly related clade that was sister to the common ancestor of *M. involucrata* and the clade of northern *M. hirsutissima* populations (Brokaw et al. 2020; Fig. 1).

This southern clade of *M. hirsutissima* corresponds in part to specimens identified as *M. hirsutissima* S.Watson var. *nesiotes* I.M.Johnst. by Rebman et al.

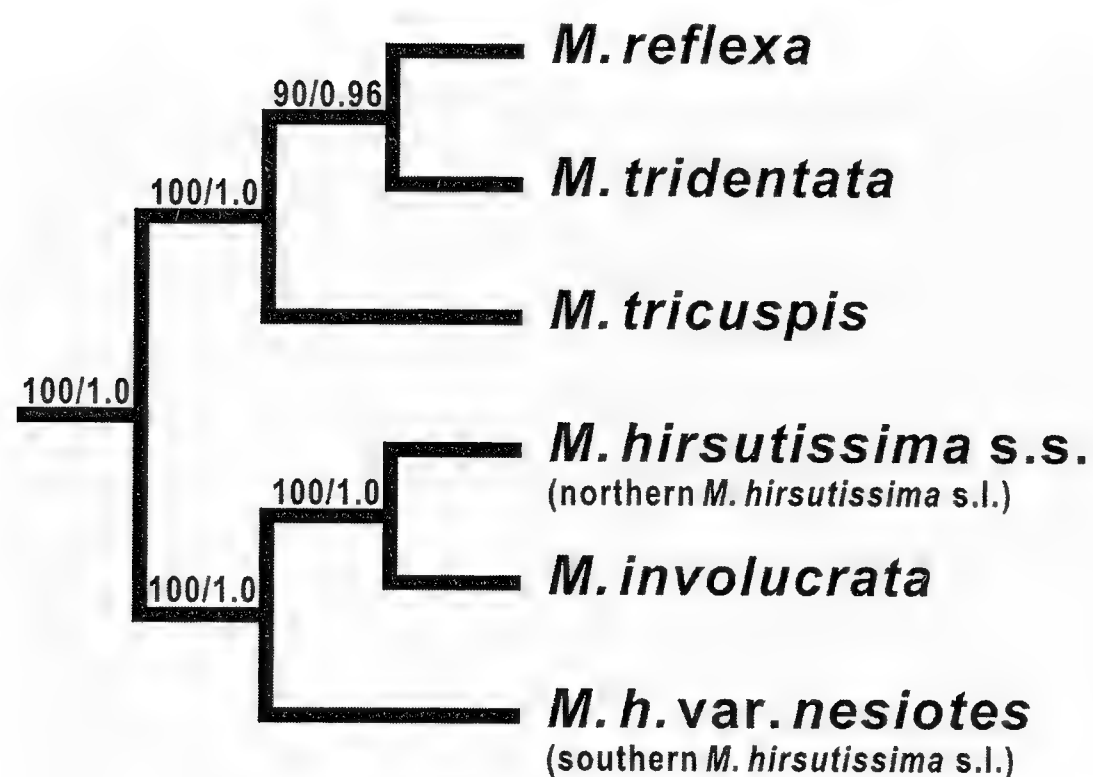


FIG. 1. Maximum likelihood phylogram of all currently recognized taxa within *Mentzelia* section *Bicuspidaria*, including *M. hirsutissima* sensu stricto (northern populations of *M. hirsutissima* sensu lato) and *M. hirsutissima* var. *nesiotes* (southern populations of *M. hirsutissima* sensu lato), based on the combined analysis of plastid (*trnL-trnF*, *trnS-trnfM*, *ndhF-rpl32*, and *rpl32-trnL* intergenic spacers) and nuclear (ITS and ETS) data sets. Branch support values represent ML bootstrap support/Bayesian posterior probability (adapted from Brokaw et al. (2020), their Fig. 2).

(2016). However, recent collections determined as *M. hirsutissima* var. *nesiotes* seem to have been based on geography rather than morphology, as many of these herbarium specimens specifically describe the flower color as yellow, which contradicts the original description of *M. hirsutissima* var. *nesiotes* as having orange flowers (Johnston 1922). Johnston (1924) later conceded that the primary morphological characters used in his protolog (1922) to distinguish the varieties of *M. hirsutissima* (flower color and stamen shape) were inconsistent and did not provide alternative characters for a diagnosis of *M. hirsutissima* var. *nesiotes*. Although the wording in the short entry is ambiguous, Johnston's rarely cited 1924 discussion of *M. hirsutissima* appears to rescind formal recognition of *M. hirsutissima* var. *nesiotes*, and more recent treatments of section *Bicuspidaria* have independently concluded that the varieties of *M. hirsutissima* were not sufficiently distinct to recognize morphological break points (Daniels 1970; Brokaw 2016).

However, given that recent phylogenetic results suggest a cryptic lineage corresponding geographically to *M. hirsutissima* var. *nesiotes*, we revisit the Mexican species of section *Bicuspidaria* to determine whether any morphological or environmental differences can distinguish the phylogenetically defined populations of *M. hirsutissima* var. *nesiotes* from the northern clade of *M. hirsutissima* (hereafter referred to as '*M. hirsutissima* sensu stricto'). As context for the findings of this investigation, we provide updated circumscriptions of the Mexican species of *Mentzelia* section *Bicuspidaria* (Fig. 2) and a key to all species in the section. We also note that this study does not substantially alter concepts published in the recent

treatment of *Mentzelia* section *Bicuspidaria* in the *Flora of North America North of Mexico* (Brokaw 2016), but it can serve as a supplement describing taxa occurring partially or entirely in Mexico.

MATERIALS AND METHODS

We obtained herbarium specimens from the following herbaria (herbaria acronyms follow Thiers [continuously updated]): California Academy of Sciences (CAS, DS), Herbario Nacional de México (MEXU), Rancho Santa Ana Botanic Garden (RSA), San Diego Natural History Museum (SD), University of California, Los Angeles (LA), and Washington State University (WS). A total of 78 herbarium specimens of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* were measured, annotated, and recorded for latitude and longitude (or coordinates were georeferenced from label data).

Morphological Comparison

We measured leaf and floral characters only from specimens that had complete sets of all characters in the following descriptions. Measurements were taken with digital calipers, using a dissecting microscope when necessary. We addressed the patterns of leaf shape and size that vary substantially with canopy position in *Mentzelia* by separately measuring leaves from the lower 1/3 and upper 1/3 of the primary axis. Leaf lengths were measured from the lamina tip to the junction of the leaf base with the stem. Leaf widths were measured at the widest point of the leaf. The intersinus width was measured as the distance between the sinuses of lobes on opposite sides of the lamina at the widest point of the lamina. Lobe width was measured at mid-length for a lobe positioned at the widest point of the leaf. The number of lobes per leaf included the total number of lobes on both sides of the leaf, excluding the terminal lobe. Bract measurements were made for the most distal bract that was adnate to a mature capsule. Bract lengths were measured from the lamina tip to the junction of the base with the capsule. Capsule measurements were made for mature capsules. Capsule lengths were measured from the end of the pedicel to the junction of the calyx. Capsule widths were measured at the widest point excluding the calyx. Calyx lobe lengths were measured from the junction with the capsule to the distal end of the calyx lobes. Petal lengths were measured from the base of the petal to its distal tip. Stamen lengths were measured from the base of the longest stamen to its distal tip. Styles lengths were measured from the distal end of the capsule to the distal end of the stigma.

Using the criteria above, we performed an exploratory study in which the following 17 characters were evaluated with linear discriminant analysis (LDA) and principal components analysis (PCA): (1) lower leaf length, (2) lower leaf width, (3) lower leaf intersinus width, (4) lower leaf lobe width, (5) lower

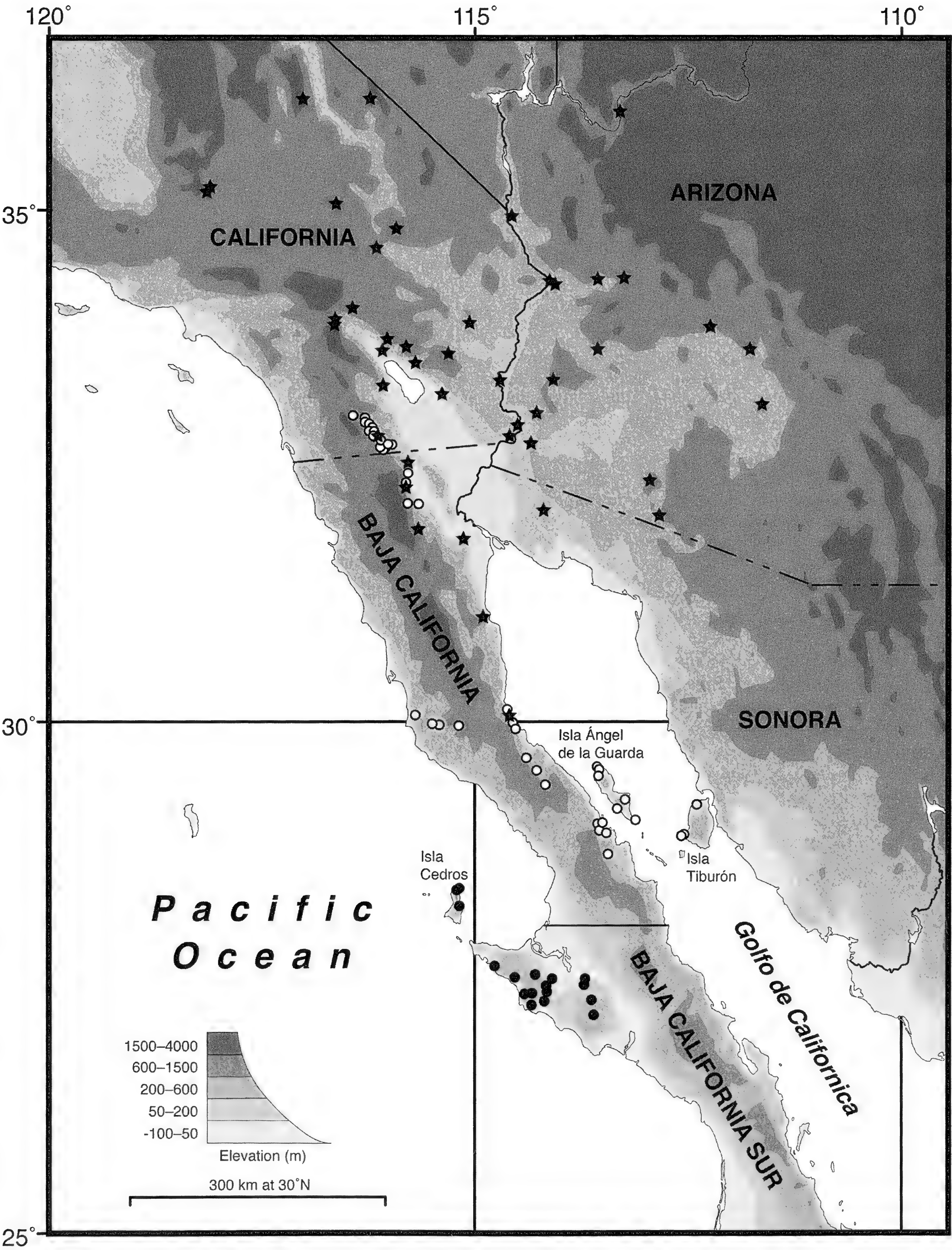


FIG. 2. Map of representative specimens. Collection locations are indicated by black stars (*M. involucrata*), open circles (*M. hirsutissima sensu stricto*), and black circles (*M. hirsutissima* var. *nesiotes*).

leaf lobe count, (6) upper leaf length, (7) upper leaf width, (8) upper leaf intersinus width, (9) upper leaf lobe width, (10) upper leaf lobe count, (11) bract length, (12) capsule length, (13) capsule width, (14) calyx lobe length, (15) petal length, (16) stamen length, and (17) style length. A combination character (the ratio of lower leaf intersinus width/lower leaf length) was also recognized and compared to the initial 17 following the selection process described below. Prior to LDA and PCA, measurements for each character were converted to Z-scores by subtraction of the mean and division by the standard deviation. We performed stepwise forward selection of characters that best discriminated between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* using LDA with CANOCO version 4.5.1 (ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003). Stepwise forward selection is a semi-automated procedure in which characters with greatest explanatory power are added to the LDA model, one at a time, until no other characters significantly (at $\alpha = 0.05$) explain residual variation in species classification of measured specimens. To test for significance, CANOCO uses the best remaining candidate character as the only discriminating character, with characters already selected included as covariables. As with other model-fitting methods, P values are determined by comparing the calculated F ratio to the expected distribution of F ratios under the null hypothesis of independence (Lepš and Šmilauer 2003). CANOCO simulates the expected distribution in a Monte Carlo permutation test by randomly reassigning values from the collected morphological measurements to the individual samples with known species designation and calculating the F ratio for each permutation replicate. The significance level (probability of type I error) of this test is then calculated as: $P = \text{number of permutations where the test statistic was not lower in the random permutation than in the analysis of original data} / \text{total number of permutations}$ (Lepš and Šmilauer 2003). Significance levels were estimated based on 999 unrestricted Monte-Carlo permutations of character data samples while holding species designations constant. PAST version 3.25 (Hammer et al. 2001) was used to perform standard methods of LDA and PCA on (1) the full data set of 17 measured characters and (2) the limited data set of characters chosen by forward selection.

To visualize character distributions by taxon, box plots that displayed the median and the four quartiles of distribution were prepared in PAST for both *M. hirsutissima* clades for: lower leaf length, the ratio of lower leaf intersinus width/lower leaf length, capsule length, and calyx lobe length. Each of these characters was evaluated for statistical difference in means by the Monte Carlo permutation test and medians by the Mann-Whitney U test with Monte Carlo permutations in PAST with 9999 permutations. The permutation test for equality of means uses the absolute difference in means as the test statistic (Hammer

2019). We treat this study as an exploratory analysis involving many explicit and implicit comparisons to construct hypotheses of morphological species concepts rather than an exercise in strict hypothesis testing. Therefore, significance levels were not corrected for multiple comparisons.

Climate Niche Comparison

We modeled the predicted current range sizes and overlap of predicted current ranges based on presence-only data using the maximum entropy algorithm in MaxEnt 3.3.3k (Phillips et al. 2006; Elith and Leathwick 2009). Location coordinates were estimated based on herbarium specimen locality descriptions (see representative specimens in the taxonomic treatment below). Uncertainty in the exact locations will result in a small amount of error in the model results presented here, but location errors are likely to fit within the 1 km² raster cell of the climate data in most cases. A challenge for narrowly distributed and rarely collected species is the relatively low sample sizes (e.g., $n = 58$ for *M. hirsutissima* sensu stricto and $n = 20$ for *M. hirsutissima* var. *nesiotes*; Fig. 2). A recent study (van Proosdij et al. 2016), however, has estimated the lower limits for sample size to be 14 for narrow-ranged taxa (e.g., *M. hirsutissima* var. *nesiotes*) and 25 for widespread taxa (e.g., *M. hirsutissima* sensu stricto) for niche estimates by MaxEnt.

To model and predict species distributions, 19 bioclimatic raster layers were obtained from WorldClim (www.worldclim.com) at 1 km² spatial resolution (Hijmans et al. 2005; Fick and Hijmans 2017). These climatic layers represent annual trends (mean annual temperature and precipitation), seasonality (annual range in temperatures and precipitation), and limiting environmental factors (temperature and precipitation of a certain quarter; Hijmans et al. 2005). Bivariate-correlation Pearson coefficients were used to identify variable pairs with correlations > 0.75 , which were subsequently removed from the analysis. Nine variables with low correlation (< 0.75) were selected: BIO1 (annual mean temperature), BIO2 (mean diurnal range), BIO3 (isothermality), BIO4 (temperature seasonality), BIO6 (minimum temperature of coldest month), BIO8 (mean temperature of wettest quarter), BIO12 (annual precipitation), BIO15 (precipitation seasonality), and BIO18 (precipitation of warmest quarter).

Seventy five percent of occurrence data were used for model training, and 25% were used for model testing with default parameters, allowing the use of linear, quadratic, product, threshold, and hinge features to produce models predicting climatic suitability under current conditions, ranging from 0–1; background points were selected randomly. The area under the receiving operating curve (AUC) was used to evaluate the accuracy of the model. Although they provide an imperfect measure of model accuracy, AUC values range from 0–1 where the AUC

TABLE 1. SUMMARY OF MORPHOLOGICAL DIFFERENCES BETWEEN *M. hirsutissima* SENSU STRICTO AND *M. hirsutissima* VAR. *nesiotes*.

Character	<i>M. hirsutissima</i> sensu stricto	<i>M. hirsutissima</i> var. <i>nesiotes</i>
Height (cm)	(5–)15–30(–40)	(3–)8–25(–35)
Lower cauline leaves (cm)	(2.9–)5.3–14.1 × 1.0–4.0	3.1–5.4(–6.7) × 0.9–2.5
Lower cauline leaf intersinus width/length ratios	0.02–0.10(–0.15)	(0.08–)0.10–0.18
Number of lower cauline leaf lobes	(7–)11–31	5–12(–14)
Calyx lobes (mm)	(12.6–)14.3–25.2 × 1.8–3.7	4.5–14.3(–17.1) × 1.8–6.1
Petals (mm)	11.9–38.7 × 11.4–35.1	13.5–24.1 × 11.9–22.1
Stamen lengths (mm)	4.0–14.0	5.0–11.5
Style lengths (mm)	(7.2–)10.1–16.1	7.5–10.4(–12.9)
Capsules (mm)	(16.7–)18.5–31.4 × 5.1–9.2	11.3–18.5(–20.9) × 4.7–7.5

values > 0.5 show the model to be better than the randomly generated model (Phillips et al. 2006; Jiménez-Valverde 2011). A jackknife test was run to evaluate the relative importance of the variables in each model. The variable with the highest gain adds the most useful information to the model, while the variable with the lowest gain importance has little effect on the model.

Ecological niche models (ENM) of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* were compared to assess their overlap with Schoener’s *D* and Hellinger-based *I* scores. Schoener’s *D* score quantified the amount of overlap between the predicted niches of the two taxa and varies between zero and one, where zero indicates no overlap and one indicates complete overlap (Warren et al. 2008). The *I* scores also measure niche similarity but are based on Hellinger’s distances, which have been standardized to vary between zero and one (Warren et al. 2008). The *D* and *I* scores were estimated in ENMTools (Warren et al. 2010) with a generalized linear model. In order to determine whether the ecological niches were more different than what we would expect by chance, we compared the niches of the two taxa with the linear range break test (Glor and Warren 2011) across 100 replicates with a generalized linear model.

In order to describe climatic differences between the niches of *M. hirsutissima* var. *nesiotes* and *M. hirsutissima* sensu stricto, we performed a PCA of the nine chosen bioclimatic variables based on data extracted for the presence points of each species. The extracted values for each bioclimatic variable were converted to Z-scores by subtraction of the mean and division by the standard deviation prior to PCA. The most important variables distinguishing the niches were determined by comparing the percentages of presence points that could be correctly discriminated according to species with each of the nine variables (not shown).

RESULTS

Morphological Comparison

Two characters used by Johnston (1922) to distinguish varieties of *M. hirsutissima* sensu lato

were noted while measuring specimens, but not used in any subsequent analyses: (1) most specimen labels of *M. hirsutissima* var. *nesiotes* and some labels of *M. hirsutissima* sensu stricto described flower color; no specimens assigned to *M. hirsutissima* var. *nesiotes* in this study indicated orange petals, whereas only two specimens assigned to *M. hirsutissima* sensu stricto indicated orange petals; (2) no specimens assigned to *M. hirsutissima* var. *nesiotes* had a middle anther-bearing stamen lobe that was shorter than the lateral lobes (all were longer or polymorphic within the flower), whereas two specimens assigned to *M. hirsutissima* sensu stricto had a middle stamen lobe that was shorter than the lateral lobes (all others were longer or polymorphic within the flower).

Linear discriminant analysis (LDA) with forward selection determined that three morphological characters provided substantial independent discriminatory power to distinguish specimens of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes*. Forward selection chose morphological characters in the order (1) capsule length ($F = 16.911$, $P = 0.001$), (2) lower intersinus width ($F = 15.009$, $P = 0.001$), and (3) calyx lobe length ($F = 4.190$, $P = 0.040$). The remaining variation in species assignment to specimens could not be significantly explained at $\alpha = 0.05$ with the available set of morphological characters. Using all three of these characters together, LDA correctly identified the species assignment of 29 of the 32 (92%) specimens.

When characters were compared individually, the ranges of measurements for *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* overlapped substantially (Table 1). Because capsule length can only be measured on specimens with mature capsules, a fourth character not chosen by forward selection with LDA was considered; lower leaf length correctly identified the same number of specimens as capsule length when used individually. However, inclusion of lower leaf length did not significantly improve species discrimination when capsule length was already included in LDA. The means permutation test and the Mann-Whitney U test identified significant differences between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* in capsule length (means difference = 5.0 mm, $P < 0.001$; $U =$

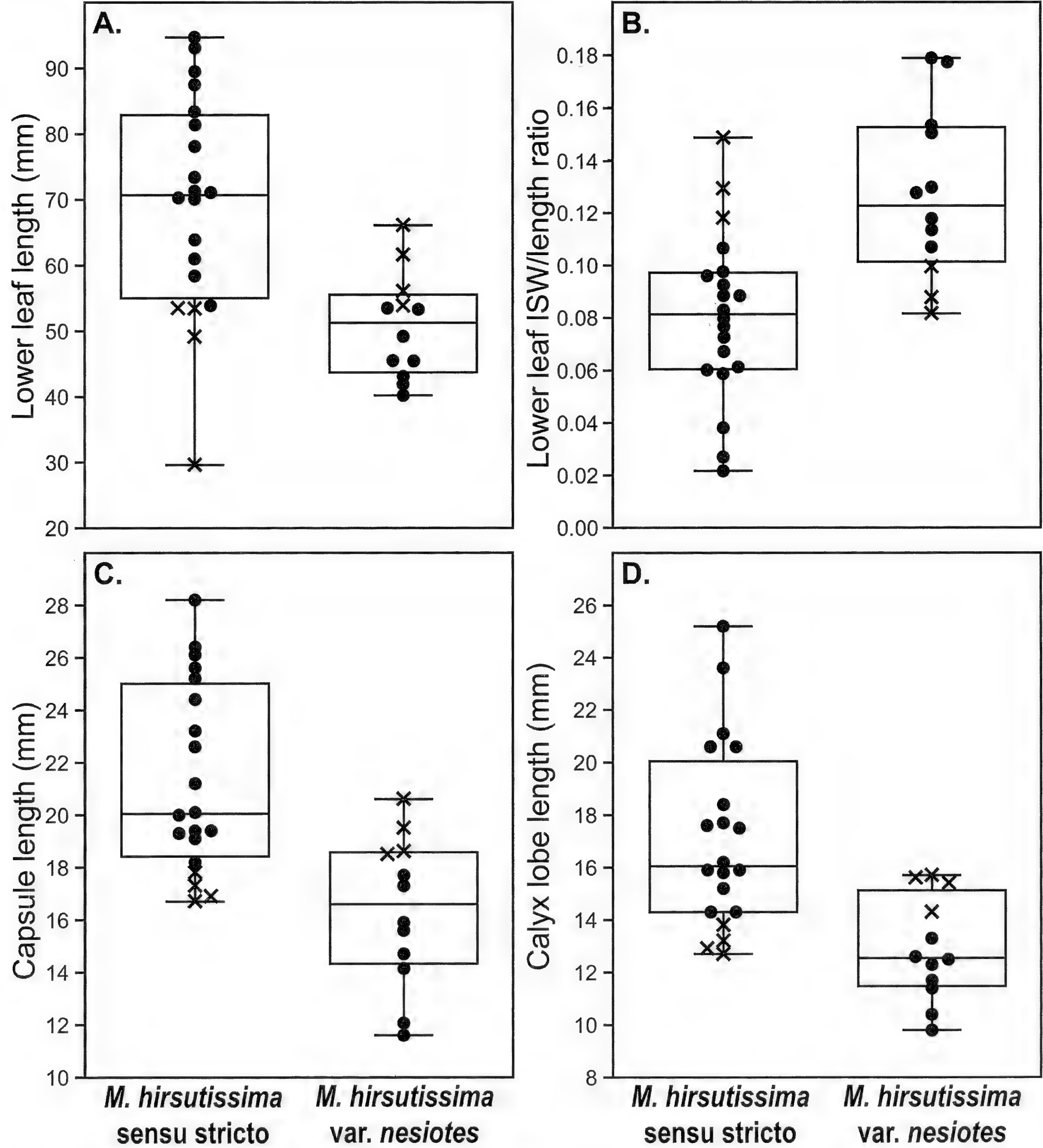


FIG. 3. Box plots of the most reliable morphological characters for discrimination between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes*. A. Lower leaf length. B. Lower leaf intersinus width (ISW)/length ratio. C. Capsule length. D. Calyx lobe length. The means permutation test and the Mann-Whitney U test found significant differences between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* for all four characters ($P \leq 0.001$). Note: box plots show median (horizontal line), first and third quartiles (boxes above and below median), and second and fourth quartiles (vertical lines); circles and X-marks represent measurements of individual specimens; X-marks indicate measurements of the respective species that were misclassified based on break-points determined by linear discriminate analysis.

35.5, $P < 0.001$), calyx lobe length (means difference = 4.2 mm, $P < 0.001$; $U = 29.0$, $P < 0.001$), and lower leaf length (means difference = 18.5 mm, $P = 0.001$; $U = 37.5$, $P = 0.001$), respectively; when used individually, each of these three characters correctly identified 24 of the 32 (75%) specimens (Fig. 3).

Lower intersinus width was not significantly different with either test (means difference = 1.1 mm, $P = 0.125$; $U = 85.0$, $P = 0.169$); when used alone, lower intersinus width correctly identified only 19 of the 32 (59%) specimens. However, when this character was combined with lower leaf length (as would be done in

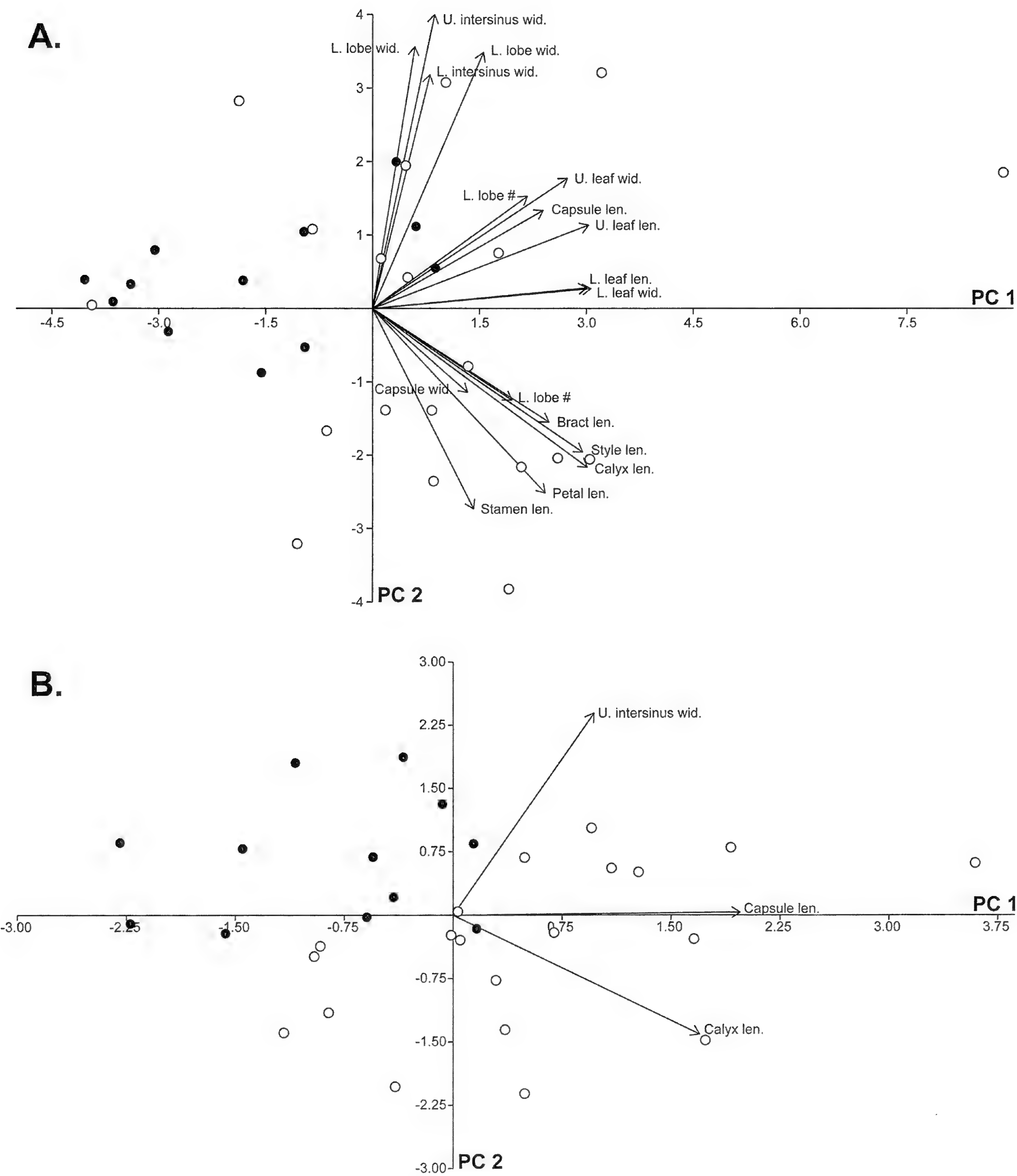


FIG. 4. Biplots for the first two principal components from principal components analysis (PCA) of measurements from A. 17 morphological characters and B. lower leaf intersinus width, capsule length, and calyx lobe length. Open circles represent *M. hirsutissima* sensu stricto, and black circles represent *M. hirsutissima* var. *nesiotes*; arrows represent direction and magnitude of loading for morphological characters on the principal component axes; U = Upper and L = Lower.

multivariate LDA and PCA comparisons) in the ratio of lower leaf intersinus width/lower leaf length, the ratio was significantly different between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* (means difference = 0.05, $P < 0.001$; $U = 35.0$, $P < 0.001$), and this composite character correctly identified 26 of the 32 (81%) specimens, more than any individual character (Fig. 3).

In the PCA derived from analysis of all 17 morphological characters, the first three PCA axes cumulatively explained 71% of the variation, with the first three axes explaining 40%, 18%, and 13% of the variation in morphological characters, respectively. The biplot of PC-1 and PC-2 displayed substantial separation between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* (Fig. 4).

TABLE 2. PERCENTAGE OF CONTRIBUTION OF BIOCLIMATIC VARIABLES USED IN MAXENT MODELS FOR *M. HIRSUTISSIMA* SENSU STRICTO AND *M. HIRSUTISSIMA* VAR. *NESIOTES*.

Variable (ordered by total contribution)	<i>M. hirsutissima</i> sensu stricto	<i>M. hirsutissima</i> var. <i>nesiotes</i>
Annual precipitation	22.6%	32.2%
Temperature seasonality	6.8%	32.8%
Isothermality	6.1%	24.2%
Precipitation of warmest quarter	22.0%	0.0%
Precipitation seasonality	10.7%	7.5%
Mean diurnal range	14.8%	0.0%
Minimum temperature of coldest month	12.2%	0.0%
Mean temperature of wettest quarter	4.7%	3.3%
Annual mean temperature	0.2%	0.0%

However, the biplot of PC-1 and PC-2 derived from only capsule length, lower intersinus width, and calyx lobe length separated specimens of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* much more effectively (Fig. 4); in this analysis, PC-1 (52% of the variation) was generally positively associated with all three characters; *M. hirsutissima* sensu stricto was associated with high values, and *M. hirsutissima* var. *nesiotes* was associated with low values of PC-1 (Fig. 4). In contrast, PC-2 (34% of the variation) was positively associated with lower intersinus width and negatively associated with calyx lobe length; *M. hirsutissima* var. *nesiotes* was associated with high values, and *M. hirsutissima* sensu stricto was associated with low values in PC-2 (Fig. 4).

Climate Niche Comparison

With respect to each other, the specimens of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* mapped in this study exhibited discrete ranges (Fig. 2). All training and test AUC values for the predicted current ranges of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* modeled by MaxEnt were high ($> 0.995 \pm$ standard deviations < 0.005), meaning they performed much better than random when predicting habitat suitability. The percentage of contributions reported for each bioclimatic variable showed that annual precipitation contributed most to the model for *M. hirsutissima* sensu stricto, whereas the model for *M. hirsutissima* var. *nesiotes* relied nearly equally on annual precipitation and temperature seasonality (Table 2).

The sizes of the predicted current niches (habitat suitability probability of > 0.2) for *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* were similar in area (ca. 9000 km²; Fig. 5). However, the predicted niche of *M. hirsutissima* sensu stricto extended across a much greater latitudinal range (ca. 600 km from north to south), whereas the compact predicted niche of *M. hirsutissima* var. *nesiotes* extended only ca. 200 km from north to south (Fig. 5). The predicted ranges (habitat suitability probability of > 0.2) of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* did not overlap (Fig. 5). Niche overlap was very low

according to Schoener’s *D* ($D = 0.11$) and Hellinger’s-based *I* ($I = 0.25$) scores; however, the linear range break test failed to determine that the niches were significantly different than what we would expect by chance ($P = 0.10$).

The predicted range of *M. hirsutissima* sensu stricto corresponded to low-to-intermediate elevations primarily east of the Peninsular Ranges of Baja California, whereas the predicted range of *M. hirsutissima* var. *nesiotes* corresponded primarily to low-to-intermediate elevations in the Vizcaino Biosphere Reserve and nearby Pacific islands west of the Peninsular Ranges (Fig. 5). Principal components analysis of the extracted bioclimatic data projected completely discrete sample distributions of *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes* in the biplot of PC-1 (37% of the variation) and PC-2 (23% of the variation; Fig. 6). *Mentzelia hirsutissima* sensu stricto was associated with greater temperature seasonality, and *M. hirsutissima* var. *nesiotes* was associated with isothermality (Fig. 6). Applied individually, the extracted values of temperature seasonality and isothermality correctly discriminated 97% and 96% of the samples according to species, respectively. Precipitation was generally lower in the habitats of *M. hirsutissima* var. *nesiotes* (7.2–12.3 cm annually) than in those of *M. hirsutissima* sensu stricto (6.8–32.8 cm annually), but only 70%–80% of the samples were correctly discriminated by individual precipitation variables used in this study.

DISCUSSION

Phylogenetic analyses by Brokaw et al. (2020) provided strong evidence that *M. hirsutissima* sensu lato is paraphyletic with respect to *M. involucrata* and, thus, does not constitute a single independent evolutionary lineage. The exploratory analyses presented here provide additional morphological, geographical, and ecological distinctions to support the recognition of *M. hirsutissima* var. *nesiotes* as a distinct species and the recircumscription of *M. hirsutissima* sensu stricto.

In 1922, Johnston described three varieties of *M. hirsutissima* sensu lato. However, Daniels (1970) and Johnston himself (1924) acknowledged that the

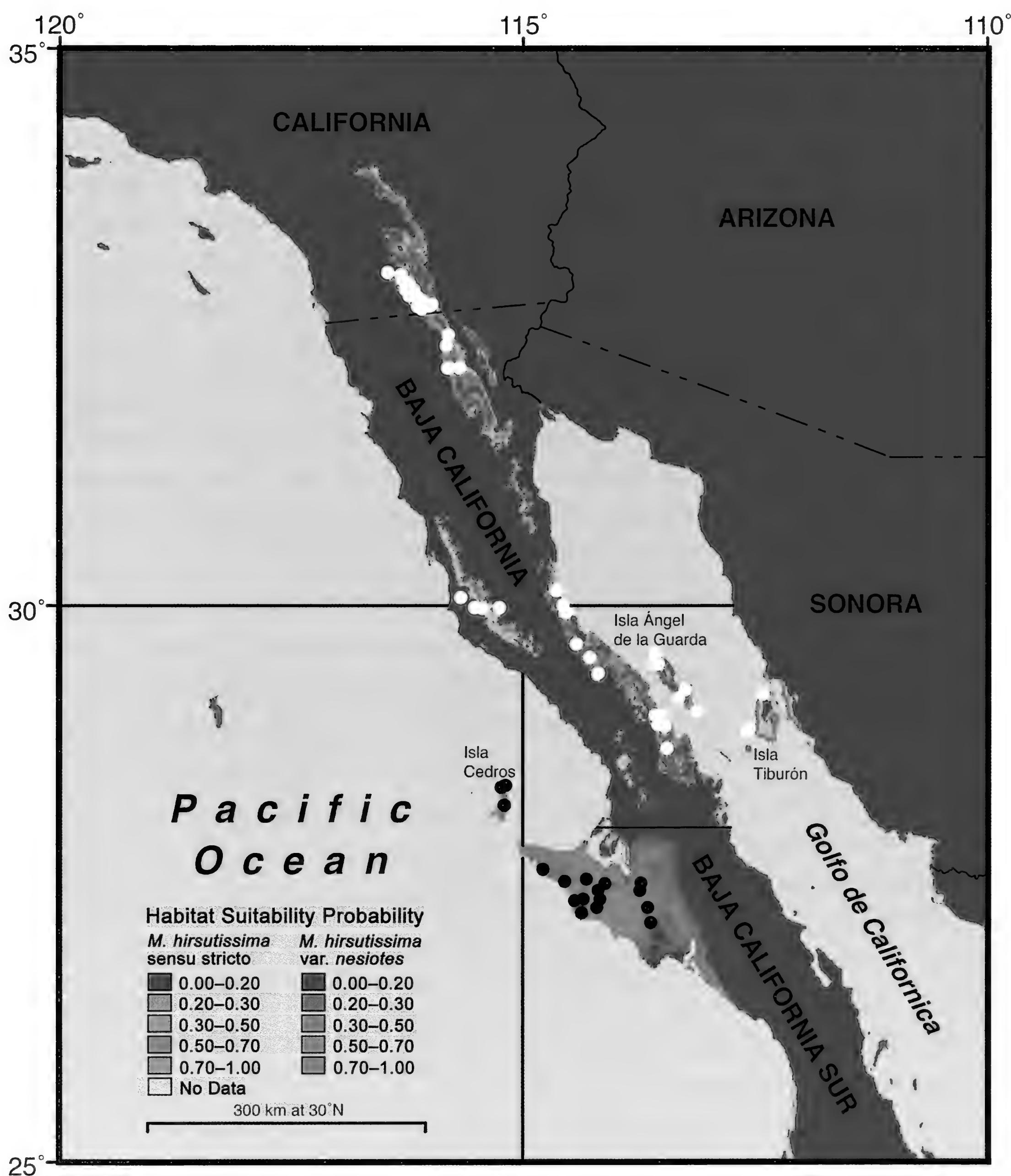


FIG. 5. Habitat suitability of *M. hirsutissima* and *M. nesiotetes* based on predictive probability habitat class estimated in MaxEnt from nine bioclimatic variables. Collection locations indicated by open circles (*M. hirsutissima*) and black circles (*M. nesiotetes*).

characters used to distinguish these varieties, including flower color and stamen shape, were dubious. Although these characters have provided consistent distinctions among some other species of *Mentzelia* (Hufford et al. 2016), perfunctory examinations of specimens in this study were sufficient to exclude flower color and stamen shape from subsequent comparisons of these taxa.

Because no discrete morphological characters have been identified to distinguish *M. hirsutissima* var. *nesiotetes* and *M. hirsutissima* sensu stricto, we employed linear discriminant analysis (LDA) and principal components analysis (PCA) to detect differences between the species within several continuous morphological characters. Although a PCA using all 17 characters only partially separated

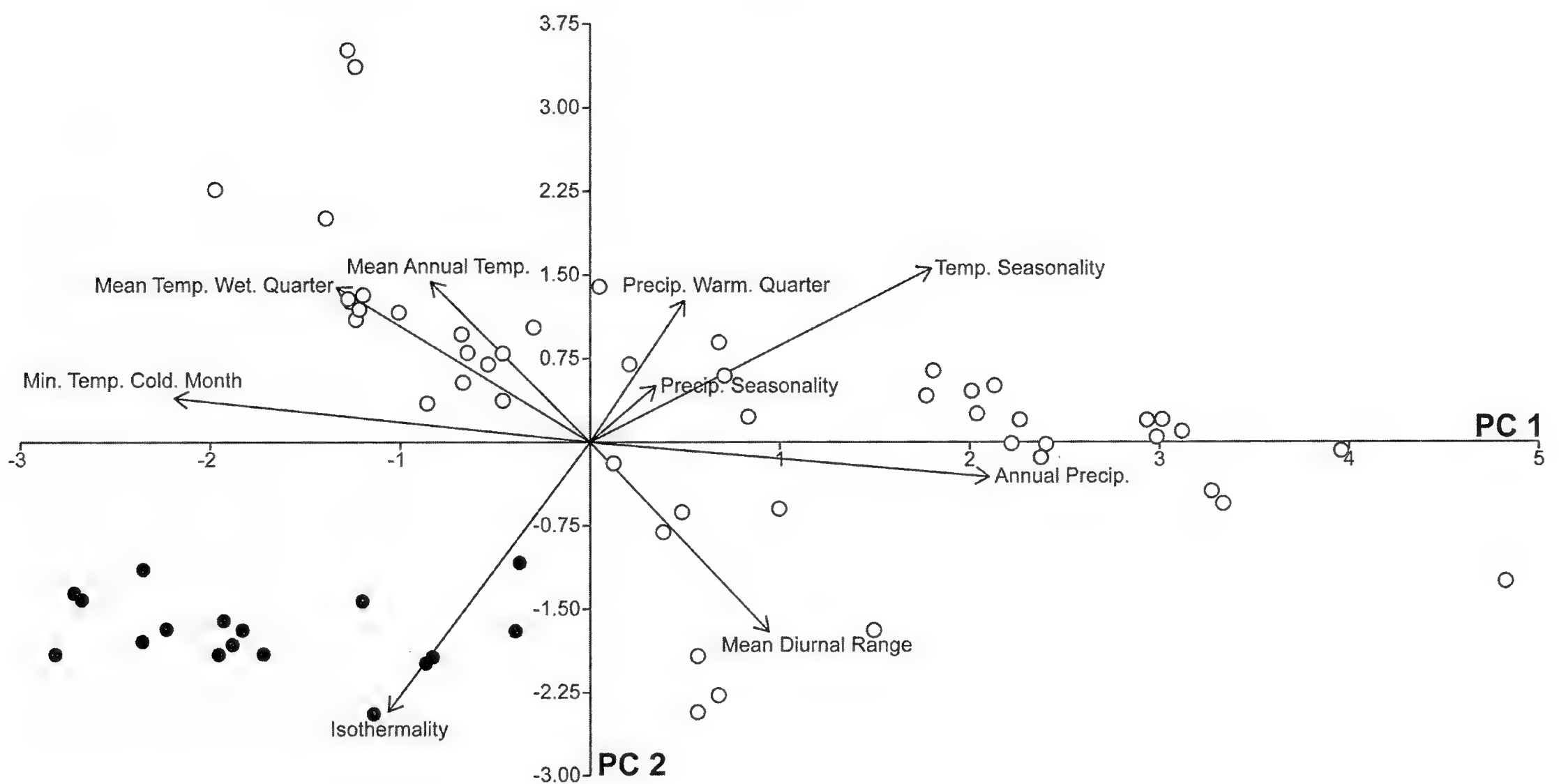


FIG. 6. Biplot for the first two principal components from principal components analysis (PCA) based on nine bioclimatic variables. Open circles represent *M. hirsutissima* sensu stricto, and black circles represent *M. hirsutissima* var. *nesiotes*; arrows represent direction and magnitude of loading for bioclimatic variables on the principal component axes.

specimens assigned to *M. hirsutissima* var. *nesiotes* and *M. hirsutissima* sensu stricto, the PCA limited to three characters (capsule length, lower intersinus width, and calyx lobe length) chosen by LDA with forward selection resulted in more discrete clustering (Fig. 4). Classification of specimens by LDA using these three characters combined resulted in a 92% success rate. The differences between the clustering of specimens in PCAs based on all 17 characters versus the three best characters can be explained by the influence of covariation among traits in the full data set as a consequence of variation in size associated with plant vigor. When characters related to overall plant size were removed from the PCA, *M. hirsutissima* var. *nesiotes* and *M. hirsutissima* sensu stricto were more successfully distinguished (Fig. 4).

Unfortunately, no single morphological character in these analyses could correctly distinguish more than 75% of the examined specimens. Nevertheless, two individual characters (capsule and calyx length) and one ratio character (ratio of lower leaf intersinus width/lower leaf length) have the greatest utility for keying specimens. Principal components analyses showed that, generally speaking, *M. hirsutissima* sensu stricto is larger than *M. hirsutissima* var. *nesiotes* in almost every morphological category (Fig. 4a; Table 1). Size differences in reproductive characters (e.g., capsule and calyx length) appear to more reliably distinguish the groups because they do not vary with canopy position and plant vigor as much as leaf size characters. When used alone, lower leaf intersinus width was a poor discriminator between *M. hirsutissima* sensu stricto and *M. hirsutissima* var. *nesiotes*. However, when leaf length

was included as a covariable by calculating the ratio of lower leaf intersinus width/lower leaf length, this ratio variable achieved a greater success rate for discrimination (81%) than any of the individual characters. Leaves of representative specimens of *M. hirsutissima* sensu stricto (Moran 12939, SD 62767, RSA 193988) and *M. hirsutissima* var. *nesiotes* (Boyd et al. 3291, RSA 500906) exhibiting contrasting ratios of intersinus width/length are illustrated in Fig. 7.

Mentzelia hirsutissima var. *nesiotes* and *M. hirsutissima* sensu stricto inhabit geographically distinct ranges, suggesting that they differ in ecological tolerances and function. Although other species of *Mentzelia* have been shown to associate with specific edaphic conditions (Schenk 2013; Hufford et al. 2016), the geographic distributions of *M. hirsutissima* var. *nesiotes* and *M. hirsutissima* sensu stricto and their proximities to mountain ranges suggest that climate could be a more significant factor. Observations by Daniels (1970) suggested that the interactions of rainfall and temperature patterns could limit geographic range through control of seed germination.

The predicted current ranges of *M. hirsutissima* var. *nesiotes* and *M. hirsutissima* sensu stricto modeled by MaxEnt are consistent with a pattern of distribution determined by climate. The estimated climate niches of *M. hirsutissima* var. *nesiotes* and *M. hirsutissima* sensu stricto have very low overlap, suggesting that these species are isolated by their climate adaptations. Populations of *M. hirsutissima* var. *nesiotes* appear to be limited to the Vizcaino Desert and adjacent Pacific islands with lower temperature seasonality, stronger isothermality, and

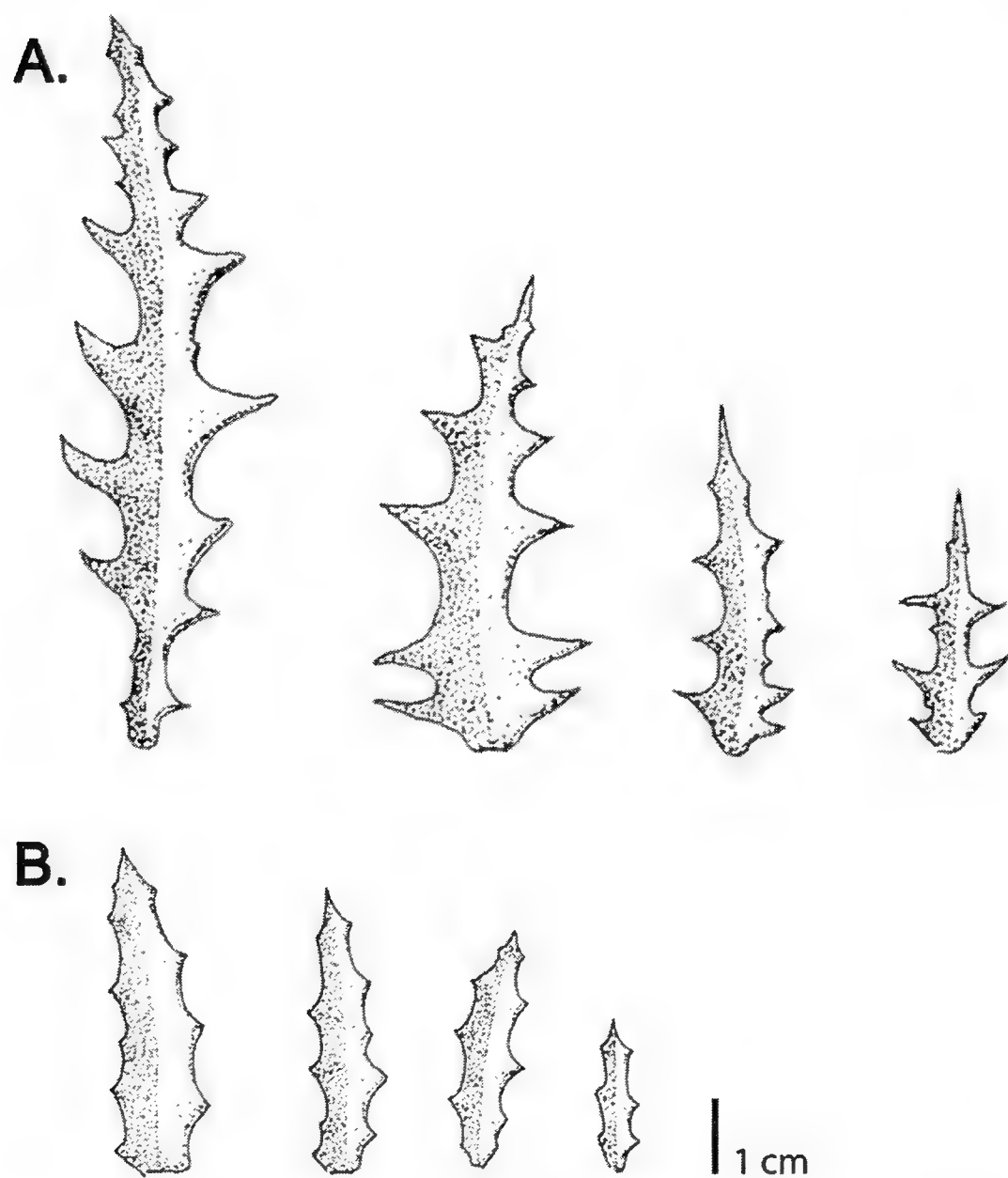


FIG. 7. Leaf comparisons between A. *M. hirsutissima* sensu stricto (based on Moran 12939, SD 62767, RSA 193988) and B. *M. hirsutissima* var. *nesiotes* (based on Boyd et al. 3291, RSA 500906). Leaves are illustrated at the same scale and arranged from the base of the plant to the apex (from left to right, respectively, with the larger leaves representing those at the base).

frequent coastal fogs, whereas populations of *M. hirsutissima* sensu stricto occur primarily in areas with greater precipitation and/or western mountain ranges that block Pacific climate influences. In fact, the only samples of *M. hirsutissima* sensu stricto that occur west of the Peninsular Ranges near El Rosario, Baja California, are closely associated with human settlements and highways, suggesting that anthropogenic disturbance and dispersal could have contributed to range extensions. Thus, the taxonomic segregation of these species is important for the recognition of ecological diversity. For example, a recent survey of plant diversity in the California Floristic Province (Burge et al. 2016) lists *M. hirsutissima* var. *nesiotes* (occurring on Cedros Island), but our current study indicates that populations of *M. hirsutissima* sensu stricto occurring in western Baja California are also inhabitants of the California Floristic Province.

An area of ongoing interest in section *Bicuspidaria* is the relationship between hybridization and chromosome numbers and comparisons of such patterns in the similar clade of annual species in section *Trachyphytum*. Molecular evidence suggests that hybridization has been common in *Trachyphytum* (Brokaw and Hufford 2010) but rare in *Bicuspidaria* (Brokaw et al. 2020). *Bicuspidaria* and *Trachyphytum* have similar numbers of diploid species, but *Trachy-*

phytum also has many polyploid complexes and no aneuploidy, whereas *Bicuspidaria* has limited aneuploidy and no known polyploidy. Thompson and Lewis (1955) showed that species with a haploid chromosome number of $n = 9$ can be found in both sections and suggested $x = 9$ as the base number of the entire genus. The solely American clade of section *Bicuspidaria*, including *M. tricuspis*, *M. tridentata*, and *M. reflexa*, has a derived $n = 10$ cytotype (Thompson and Roberts 1971), whereas the only previously published chromosome count for the Mexican clade is $n = 9$ for *M. involucrata* (Thompson and Lewis 1955). However, we report here that a specimen of *M. hirsutissima* sensu stricto (H. J. Thompson 3688, LA accession 38630; $n = 9$) in the UCLA Herbarium documents chromosome counts performed by H. J. Thompson and M. Poston following the methods of Thompson and Lewis (1955; Poston, Pitzer College, personal communication). Future work should prioritize acquisition of a count for *M. hirsutissima* var. *nesiotes*, but the most parsimonious inference would be that *M. hirsutissima* var. *nesiotes*, like *M. hirsutissima* sensu stricto and *M. involucrata*, bears the ancestral $n = 9$ cytotype. Under this assumption, species in section *Bicuspidaria* with greatest morphological similarity and geographic proximity also have the same number of chromosomes. Therefore, cytotype differences do not explain the cryptic phylogenetic diversity or why geographically close species do not appear to hybridize in section *Bicuspidaria*. Instead, adaptation to distinct, albeit similar, habitats seems to have contributed to greater reproductive isolation in section *Bicuspidaria*, often without substantial divergence of morphological characters. Furthermore, this study emphasizes that cryptic morphology can mask important ecological differences with significance for conservation and ecosystem function and that a total-evidence approach involving morphological, geographical, physiological, and several sources of genetic data maximizes our understanding of phylogenetic and ecological diversity.

TAXONOMIC TREATMENT

Mentzelia hirsutissima S. Watson, Proc. Amer. Acad. Arts 12: 252. 1877. *Bicuspidaria hirsutissima* (Watson) Rydberg, Bull. Torrey Bot. Club 30: 275. 1903. —Type: Mexico, Baja California: Mun. Ensenada, Feb 1876, T. H. Streets s.n. (lectotype [here designated]: GH 76070!; isolectotypes: GH 76069! US 55961!). Fig. 7.

Mentzelia stenophylla Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 80. 1900. *Mentzelia hirsutissima* var. *stenophylla* (Urb. & Gilg) I.M. Johnst., Univ. Calif. Publ. Bot. 71: 443. 1922. —Type: Mexico, Baja California: Mun. Ensenada, 22 Apr 1886, C. R. Orcutt 1357 (holotype: NY 112243!; isotype: GH 76073!).

Mentzelia piersonii Jepson, A Flora of California 2: 529, fig. 248. 1936. —Type: USA, California:

Imperial Co., 17 Apr 1927, *W. L. Jepson 11764* (holotype: JEPS 2647!; isotype: RSA 4124).

Annual herbs, (5–)15–30(–40) cm tall; taprooted. Shoots densely pubescent throughout (except as noted) with both needle-like trichomes with erect barbs and glochidiate trichomes with recurved barbs arranged in whorls. Stem erect; axillary branches ascending, straight to curved upward; stem epidermis light tan to salmon-colored, moderately pubescent. Leaves alternate, pubescent; basal rosette leaves $0.5\text{--}7.1 \times 0.3\text{--}1.9$ cm, oblanceolate, sessile or appearing petiolate due to narrowed lamina at base, often not persisting to maturity, margins entire to shallowly lobed, intersinus width 1.3–9.0 mm, lobes $0.8\text{--}7.9 \times 0.6\text{--}3.1$ mm with rounded to acute apices; lower cauline leaves $2.9\text{--}14.1 \times 1.0\text{--}4.0$ cm, lanceolate, appearing petiolate to clasping, margins 7–31-lobed, intersinus width 1.8–14.5 mm, lobes $4.5\text{--}26.5 \times 1.1\text{--}8.5$ mm with rounded to acute apices; upper cauline leaves $2.5\text{--}7.7 \times 0.8\text{--}4.2$ cm, ovate to lanceolate, base often cordate-clasping, margins 6–21-lobed, intersinus width 1.6–15.5 mm, lobes $3.9\text{--}27.4 \times 0.6\text{--}5.2$ mm with rounded to acute apices. Inflorescence cymose, bract adnate to inferior ovary, $12.1\text{--}30.2 \times 5.3\text{--}10.1$ mm, ovate, margins 5–9-lobed, green, not concealing pedicel or ovary. Flowers epigynous; bearing a hypanthium at the distal end of the ovary on which the calyx, corolla, and androecium are inserted. Calyx basally connate, five lanceolate lobes, lobes $12.6\text{--}25.2 \times 1.8\text{--}3.7$ mm, apices acute to attenuate, margins entire, pubescent; trichomes like those of leaves. Petals five, distinct, $11.9\text{--}38.7 \times 11.4\text{--}35.1$ mm, obovate, cream-colored to yellow (rarely orange) with veins more deeply pigmented, glabrous, apex mucronate. Androecium cream-colored; stamens ca. 15–40, ca. 4.0–14.0 mm; filaments \pm monomorphic, linear, most or all distally 2-lobed with orange lateral band inferior to distal lobes, lobes to 0.5 mm; anther stalk usually longer than filament lobes, glabrous; anther epidermis papillate. Gynoecium 3-carpellate; ovaries inferior, placentae 3, parietal; styles 7.2–16.1 mm long, terete, glabrous; stigmas three, lobes appressed, papillate. Fruit a capsule, $16.7\text{--}31.4 \times 5.1\text{--}9.2$ mm, cylindrical, tapering towards the base with prominent longitudinal ribs, dehiscing septicidally by three valves, pubescent; trichomes like those of leaves. Seeds in two series, ca. 2.5×1.5 mm, pyriform (widest at middle), flattened, rugose; seed coat pale grey; seed coat cells 20–30 μm wide. Chromosome number $n = 9$ (here reported).

Populations of *M. hirsutissima* occur primarily on rocky or sandy soils in arroyos, canyons, badlands, and mountain slopes in three subranges within southern California and northern Baja California, including: (1) eastern slopes of the Peninsular Ranges extending approximately 50 km north to 50 km south of the California-Baja California border, (2) eastern slopes of the Peninsular Ranges and Gulf of California islands extending approximately 130 km north to 30 km south of the type locality at Isla

Ángel de la Guarda, and (3) western slopes of the Peninsular Ranges near El Rosario, Baja California (Fig. 2). Collections of *M. hirsutissima* have been reported at 0–700(1100) m elevation. *Mentzelia hirsutissima* is listed by the California Native Plant Society as a California Rare Plant Rank 2B.3: plants rare in California, common elsewhere, not very endangered in California (California Native Plant Society 2020). *Mentzelia hirsutissima* begins flowering in mid-February and continues through mid-May. Plants bearing both flowers and ripe capsules with mature seeds are most common in April. By late May, most plants have senesced and disintegrated. See discussion below that details the sympatric populations of *M. hirsutissima* and *M. involucrata*.

Johnston (1924) and Daniels (1970) independently concluded that the characters originally purported by Johnston (1922) to distinguish *M. hirsutissima* var. *stenophylla* from *M. hirsutissima* var. *hirsutissima* are not consistent across or within populations. However, Daniels (1970) recommended that they be synonymized (in an unpublished dissertation), whereas Johnston (1924) maintained that *M. hirsutissima* var. *stenophylla* should continue to be recognized. Johnston proposed that *M. hirsutissima* var. *hirsutissima* was known only from the type locality and that *M. hirsutissima* var. *stenophylla* was a “small-flowered form” of the species. Nevertheless, our morphological analyses have discerned no clear discontinuities in flower size, and phylogenetic analyses by Brokaw et al. (2020), including specimens from the type locality and those cited by Rebman et al. (2016) as possible examples of *M. hirsutissima* var. *hirsutissima* and *M. hirsutissima* var. *stenophylla*, identified no distinctions. We, therefore, reduce *M. hirsutissima* var. *stenophylla* to synonymy with the species.

Representative specimens (Note: latitude, longitude, and/or elevation that are estimated from specimen label locality information are indicated with an asterisk (*); otherwise, these are transcribed from label information. See Fig. 2 for map of localities). MEXICO. BAJA CALIFORNIA. **Mun. Ensenada:** Puerto Refugio, N end of Isla Ángel de la Guarda, 29.5333*, –113.5583*, 10* m, 18 Apr 1962, *Copp 129* (DS 493462, LA 48303); Bahía de Los Angeles, S shore, along inner road W of La Mona, near rear of house on S side of road, 28.9992*, –113.5746*, 0* m, 13 Mar 1992, *Fritsch 1289* (RSA 543236); Los Flores, Los Angeles Bay, 28.9500*, –113.5600*, 0* m, 11 Apr 1947, *Harbison 41722* (DS 325653, SD 41722); San Fernando Mission, 29.9783*, –115.2006*, 500* m, 13 Apr 1954, *Harvey s.n.* (SD 45665); Opposite Pond Island on silty flats, 29.0333*, –113.1333*, 0* m, 30 Jun 1921, *Johnston 4229* (CAS 48173); Isla Ángel de la Guarda, SE corner, near Pond Island, 29.0333*, –113.1333*, 10* m, 6 Apr 1947, *Lindsay s.n.* (DS 428693); Isla Ángel de la Guarda, first large canyon W of Punta Diablo (= Rock Point), 29.2333, –113.2500, 200 m, 24 Mar 1959, *Moran 7238* (SD 49611; MEXU 22234); Isla

Ángel de la Guarda, Arroyo Estaton, 29.1417, -113.3417, 20 m, 15 Apr 1960, *Moran 8179* (SD 52821); Isla Ángel de la Guarda, Arroyo Estaton, 29.1417, -113.3417, 100 m, 15 Mar 1962, *Moran 8578* (SD 61547); Isla Ángel de la Guarda, first cove E of Puerto Refugio, 29.5333, -113.5583, 150 m, 16 Mar 1962, *Moran 8631* (LA 53257, RSA 207502, SD 61559); Mejia Island, (near) Isla Ángel de la Guarda, 29.5583, -113.5833, 10 m, 23 Mar 1963, *Moran 10487* (SD 54135); Rocky hillside above Bahía de Los Angeles, 28.9323, -113.5534, 200 m, 4 Mar 1966, *Moran 12426* (LA 87918, SD 64924); 2 miles N of Las Venecas, 28.7000, -113.4500, 270* m, 10 Mar 1966, *Moran 12466* (DS 598318, LA 87922, SD 64922); Isla Ángel de la Guarda, arroyo 5 miles S of Puerto Refugio, 29.4688, -113.5740, 50 m, 20 Apr 1966, *Moran 12893* (LA 87919, SD 62770); Isla Ángel de la Guarda, colony on S slope, summit of Cerro Angel, 29.4663, -113.5501, 1100 m, 20 Apr 1966, *Moran 12916* (DS 598795, LA 87942, SD 62769); Mejia Island, 29.5527, -113.5745, 10 m, 21 Apr 1966, *Moran 12939* (SD 62767, RSA 193988); Isla Ángel de la Guarda, Pond Island Bay, 29.0333, -113.1333, 25 m, 22 Apr 1966, *Moran 12974* (LA 87921, SD 62768); 7.5 miles SE of Okie Landing, 29.9333, -114.5333, 50 m, 4 Apr 1970, *Moran 17263* (SD 77008); Back of beach, SE part of Bahía de Los Angeles, 7.5 miles from town, 28.9081*, -113.4713*, 10* m, 27 Mar 1960, *Porter 569* (CAS 437677, LA s.n.); NE-SW valley 20.6 miles E of El Rosario on Hwy 1, 30.0658, -115.7182, 430 m, 11 May 1983, *Prigge 4605* (LA 102776, RSA 328771); Wash 35 km S of Puertocitos, near El Huerfanito, 30.1267, -114.6333, 20 m, 6 Mar 1988, *Prigge 8021* (LA 104895); E-facing canyon 24.6 km NW of Punta Willard and 0 to 3 km W of road, 29.9917, -114.5667, 40–100 m, 7 Mar 1988, *Prigge 8040* (LA 104926); Rancho Aguajito, 29.9833, -115.5167, 300 m, 22 Apr 1958, *Raven 12673* (LA 30538); Mejia Island, 29.5550*, -113.5660*, 10* m, 2 Apr 1973, *Thompson 3688* (LA 38630); 18 miles S of Bahía San Luis Gonzaga Resort, 29.5207*, -114.2925*, 450* m, 5 Jun 1966, *Verity s.n.* (LA 83654); Calamejue Canyon, 29.3833, -114.1833, 300–400 m, 26 Mar 1973, *Webster 18174* (SD 96009); Bahía de Los Angeles, Bota Island, above SW beach, 29.0100, -113.5150, 10* m, 10 Mar 1999, *West 99-105* (SD 145677); 6 miles SW of S end of Bahía de San Luis Gonzaga, 29.6483*, -114.4133*, 230* m, 24 Mar 1960, *Wiggins 15913* (DS 513917); Bahía de Los Angeles, 28.9323, -113.5530, 30–90 m, 12 Feb 1962, *Wiggins 220* (DS 629793); Side canyon of Arroyo Grande E of El Rosario, near “end of road” N of San Juan de Dios, 29.9681*, -115.4359*, 310* m, 2 May 1993, *Wisura et al. 4858* (MEXU 684284, RSA 554542). **Mun. Mexicali:** On E slope along the trail that enters Cantiles Canyon from El Progreso, 32.1500, -115.8000, 990 m, 3 Apr 1966, *Beauchamp s.n.* (SD 64003); Sands of bajada from Cantu Palms, 32.3500, -115.8300, 100 m, 8 Mar 1987, *Clemons 1601* (SD 120943); Guadalupe Canyon, 32.1445, -115.6835, 500 m, 12 Mar 1988, *Clemons 1992* (SD

122252); NE-facing slope between Laguna Salada & Sierra Juárez, 32.4467, -115.8067, 55 m, 1 Apr 1992, *Clemons 2365* (SD 133327); Guadalupe Canyon, E side of Sierra Juárez, 32.1500, -115.8167, 200 m, 6 May 1957, *Moran 6031* (DS 440764, RSA 138463, SD 48881); Below Cantu Palms on E base of Sierra de Juárez, ca. 15 mi S of jct of Baja California Hwy 2 and Guadalupe Canyon Road, 32.3517*, -115.8276*, 100* m, 22 Mar 1986, *Thorne 61630* (LA 103806, MEXU 720847, RSA 350002); Guadalupe Canyon, along canyon above resort area, 32.1452, -115.8000, 400 m, 23 Mar 1986, *Thorne 61749* (RSA 349150); Guadalupe Canyon, along canyon above resort area, 32.1500, -115.8000, 500 m, 23 Mar 1986, *Thorne 61791* (LA 103804). **SONORA. Mun. Hermosillo:** Isla Tiburón, vicinity of Tecamate, Bahía Agua Dulce, NW part of island, 29.1855, -112.4119, 10* m, 21 Mar 1963, *Felger 6815-B* (ARIZ 337799); Canyon just N of Willard Point, Tiburón Island, 28.8762, -112.5785, 10* m, 19 Mar 1962, *Moran 8722* (LA 53253, SD 66256); Willard Point, Gonzaga Bay, 28.8762*, -112.5784*, 30* m, 23 Mar 1937, *Rempel 284a* (RSA 445116); W side of Isla Tiburón, just N of Punta Willard, 28.8844*, -112.5571*, 10* m, 19 Mar 1962, *Wiggins 17154* (DS 513122). **USA. CALIFORNIA. Imperial Co.:** Jacumba Mountains, to the N of Meyer Valley, approximately 3.5 air miles NE of Mountain Spring, 32.6927, -116.0550, 423 m, 5 May 2015, *Bell 8185* (RSA 101327); Foot of Mountain Springs Grade, western Imperial County, 32.6771*, -116.0971*, 650* m, 16 Apr 1927, *Peirson 7209* (RSA 69555); 6.5 miles W of Ocotillo or 3.5 miles E of Devil's Canyon, 32.7256*, -116.0398*, 250* m, 16 Feb 1964, *Turner 1381* (RSA 271698); Roadside, Yuha Cut-Off, 1 mile S of Highway 8, 32.7248, -115.9929, 700 m, 1 Mar 1970, *Witham 671* (SD 73532). **San Diego Co.:** Anza-Borrego State Park, 1 mile N of Agua Caliente on S2, on right/N side of road is marked trail called Bismaga Wash, Square M25, 32.9850, -116.3178, 404 m, 6 Mar 2005, *Angel 147* (SD 173880); Along N drainage from Inner Pasture, Agua Caliente quad, 32.9312, -116.2762, 400* m, 7 Apr 1992, *Clemons 2384* (SD 133328); Lower Box Canyon, 33.0137*, -116.4524*, 720* m, 29 Apr 1941, *Gander 9187* (SD 28612); Anza-Borrego Desert, South Canebrake above and in the wash that borders the State Park, 32.8989, -116.2411, 320* m, 27 Feb 2005, *Glacy 16* (SD 177090); Anza-Borrego Park, Indian Canyon, 3.2 air miles WSW of intersection of County Road S-2 and access road to canyon, 32.8595, -116.2630, 452 m, 31 Mar 2008, *Hendrickson 2857* (SD 214424); Anza-Borrego Park, Rockhouse Canyon, 2.7 miles S of intersection of County Road S-2 and Carrizo Wash jeep trail, 32.8096, -116.2139, 292 m, 30 Mar 2009, *Hendrickson 3614* (SD 214425); Agua Caliente Regional Park, Marsh (Squaw) Canyon, 0.5 mile SW of intersection of County Road S-2 and access road to park, Square M26, 32.9533, -116.3090, 418 m, 20 Mar 2010, *Hendrickson 4526* (SD 219916); SE of Agua Caliente, Anza Borrego Park, 32.9322*,

–116.2591*, 320* m, 11 Mar 1977, *Howell 52340* (CAS 170811); Anza-Borrego Park, Mountain Palm Springs, 500 yds SW of intersection with S-2 near parking lot, 32.8634, –116.2129, 270 m, 7 Mar 2008, *Nenow 412* (SD 212285); Scraped roadside about 1.5 miles S of turnoff to Canebrake on S-2, 32.8950*, –116.2280*, 270* m, 16 Apr 1978, *Oberbauer 10* (SD 99513); Anza-Borrego Park, SW of Highway S-2 and S of Dos Cabezas, near the San Diego/Imperial County line, 32.7010, –116.1276, 615 m, 10 Apr 2009, *Rebman 16852* (SD 193161); Anza-Borrego Park, W of Sweeny Pass Rd. (S2) between Canebrake and Ocotillo, along Mortero Canyon Road, 32.7703, –116.1256, 390 m, 17 Mar 2017, *Rebman 32483* (SD 262088). Note: LA, MEXU, and RSA specimens of *Thorne 61630* are mixed collections of *M. hirsutissima* and *M. involucrata*.

Mentzelia involucrata S.Watson, Proc. Amer. Acad. Arts 20: 367. 1885. *Bicuspidaria involucrata* (Watson) Rydberg, Bull. Torrey Bot. Club 30: 275. 1903. *Nuttallia involucrata* (S.Watson) Davidson & Moxley, Fl. S. Calif. 240. 1923, where incorrectly attributed to S. Watson. —Type: USA, California: San Bernardino Co., 1876, *C. C. Parry and J. G. Lemmon 138*, (lectotype [designated by Darlington 1934]: US 1339132!; isoelectotypes: GH 76047!, NY 112203!, NY 112204!, NY 621937!, NY 621939!); syntype: USA, Arizona: Maricopa Co., Wickenburg, Apr 1876, *E. Palmer 598* (GH 76048!; isosyntypes: NY 112202!, NY 563859!, NY 563860!, UC 108386, YU 65383!); syntype: USA, Arizona: Mohave Co., Yucca, May 1884, *M. E. Jones 100* (GH 76049!; isosyntypes: POM 83504, POM 84218, POM 84232); syntype: Mexico, Sonora: Caborca Mun., Port Lobos, 28 Mar 1884, *C. G. Pringle 46* (GH 76071!; isosyntypes: F 90815!, NY 112240!, US 55955!, US 1339130!).

Mentzelia involucrata S.Watson var. *megalantha* I.M.Johnst., Univ. Calif. Publ. Bot. 71: 443. 1922. *Nuttallia involucrata* (S.Watson) Davidson & Moxley var. *megalantha* Munz & I.M.Johnst., Fl. S. Calif. 240. 1923. —Type: USA, California: Riverside Co., 21 Apr 1905, *J. G. Hall 5839* (holotype: UC 68744!).

Annual herbs, 7–35(–45) cm tall; taprooted. Shoots densely pubescent throughout (except as noted) with both needle-like trichomes with erect barbs and glochidiate trichomes with recurved barbs arranged in whorls. Stem erect; axillary branches ascending, straight to curved upward; stem epidermis light tan to salmon-colored, moderately pubescent. Leaves alternate, pubescent; basal rosette leaves 0.9–8.1 × 0.4–2.1 cm, oblanceolate, sessile or appearing petiolate due to narrowed lamina at base, often not persisting to maturity, margins entire to shallowly lobed, intersinus width 2.3–17.0 mm, lobes 0.3–4.9 × 0.5–3.3 mm with rounded to acute apices; lower cauline leaves 2.9–16.3 × 1.0–4.1 cm, lanceolate, appearing petiolate to clasping, margins 7–33-lobed,

intersinus width 4.3–28.5 mm, lobes 2.5–23.5 × 1.1–7.5 mm with rounded to acute apices; upper cauline leaves 2.3–7.8 × 1.0–4.3 cm, ovate to lanceolate, base often cordate-clasping, margins 7–25-lobed, intersinus width 4.6–24.5 mm, lobes 2.3–9.4 × 0.6–5.2 mm with rounded to acute apices. Inflorescence cymose, bract adnate to inferior ovary 13.1–27.2 × 10.3–24.1 mm, broadly obovate, margins 9–13-lobed, white with green margins, conspicuous, concealing pedicel, and ovary. Flowers epigynous; bearing a hypanthium at the distal end of the ovary on which the calyx, corolla, and androecium are inserted. Calyx basally connate, five lanceolate lobes, lobes 12.6–31.2 × 2.2–5.1 mm, apices acute to attenuate, margins entire, pubescent; trichomes like those of leaves. Petals five, distinct, 13.1–65.3 × 11.4–55.1 mm, obovate, white to pale yellow with yellow to orange veins, glabrous, apex mucronate. Androecium white to cream-colored; stamens ca. 15–40, ca. 4.0–26.0 mm; filaments ± monomorphic, linear, most or all distally 2-lobed with orange lateral band inferior to distal lobes, lobes 0.5–2.0 mm; anther stalk shorter or longer than filament lobes, glabrous; anther epidermis papillate. Gynoecium 3-carpellate; ovaries inferior, placentae 3, parietal; styles 8.0–30.1 mm long, terete, glabrous; stigmas three, lobes appressed, papillate. Fruit a capsule, 14.5–30.4 × 5.1–10.2 mm, cylindrical, tapering towards the base with prominent longitudinal ribs, dehiscing septicidally by three valves, pubescent; trichomes like those of leaves. Seeds in two series, ca. 2.5 × 2.0 mm, widest at middle, constricted proximal to distal 1/4, flattened, rugose; seed coat pale grey; seed coat cells 25–35 µm wide with wavy anticlinal cell walls. Chromosome number $n = 9$ (Thompson and Lewis 1955).

Mentzelia involucrata is the most northern, most widely distributed, and most frequently collected of the species in this treatment with only a minority of its populations occurring in Mexico (Fig. 2). Populations occur in rocky areas and arroyos throughout the Colorado Desert extending south to Bahia San Luis Gonzaga in Baja California; east into Pinal County, Arizona; north into Inyo County; and west into Kern County, California. Collections of *M. involucrata* have been reported at 0–1300 m elevation. *Mentzelia involucrata* has not been listed as rare, threatened, or endangered. *Mentzelia involucrata* begins flowering in mid-January and continues through mid-May. Plants bearing both flowers and ripe capsules with mature seeds are most common in mid-March through mid-April. By mid-May, most plants have senesced and disintegrated. Although sympatry among species in section *Bicuspidaria* is uncommon (Daniels 1970), mixed populations of *M. involucrata* and *M. hirsutissima* sensu stricto may occur in far southern California and northern Baja California at the southern end of the *M. involucrata* distribution and the northern end of the *M. hirsutissima* sensu stricto distribution. Despite the presence of conspicuous white bracts distinguishing *M. involucrata* from other species in *Bicuspidaria*,

mixed collections of *M. involucrata* and *M. hirsutissima* sensu stricto have been identified, as noted below.

Mentzelia involucrata var. *megalantha* was described as having petals 3.5–4.5 cm long as opposed to *M. involucrata* var. *involutrata*, which has petals 1.5–2.5 cm long (Johnston 1922). Daniels (1970) performed thorough quantitative analyses and determined that: (1) flower size is distributed continuously in *M. involucrata*, (2) flower size generally correlates with plant size, and (3) the geographical distribution of flower sizes suggests environmental and/or genetic clines for flower size. Because the largest flowered populations of *M. involucrata* occur towards the center of its geographic distribution (Daniels 1970), we suggest that specimens that are best adapted to their habitats are most vigorous and tend to create the largest flowers. Furthermore, the phylogenetic analysis of Brokaw et al. (2020) found no clear distinction between specimens keyed to *M. involucrata* var. *megalantha* versus *M. involucrata* var. *involutrata*. Therefore, we reduce *M. involucrata* var. *megalantha* to synonymy with the species.

The status of the lectotype and syntypes of *M. involucrata* has been confusing and not fully explained previously. Daniels (1970), in his unpublished Ph.D. dissertation, suggested that Darlington (1934) had incorrectly lectotypified *M. involucrata* because the specimen she listed as the ‘Type’ (Parry and Lemon 138, US 1339132) had not been examined or listed in the protolog by Watson (1885). Holmgren et al. (2005), however, determined that the lectotype had been selected by Darlington inadvertently, but effectively. Darlington’s lectotypification of US 1339132 appears to have been based in part on a misplaced annotation. US 1339132 was originally identified as *M. tricuspis* by Parry in 1876 (prior to the description of *M. involucrata* by Watson in 1885), and it was first annotated as a collection of *M. involucrata* by Urban and Gilg in 1892 (and never annotated by Watson). However, an undated annotation attributed to Parry incorrectly identifies US 1339132 as a ‘type-specimen’ for *M. tricuspis* Gray (Parry had collected the true holotype of *M. tricuspis*, GH 76058, two years earlier in 1874), and this ‘type’ designation, despite applying to a different species, was transcribed by Darlington (1934) into her treatment of *M. involucrata*. Nevertheless, although this lectotype was probably never examined by Watson, we suggest that it does not meet the criterion of ‘serious conflict’ with the protologue necessary to be superseded. Watson (1885) originally designated four syntypes, presumably housed at GH where he was curator. Originals, or perhaps duplicates, of each of these syntypes remain at GH, but Watson’s protolog does not indicate a specific herbarium for the syntypes, making the discrimination between syntypes and isosyntypes ambiguous. Watson described one of his syntypes as “In San Bernardino County, California, by C. C. Parry in 1876;” this text is identical to the label of GH 76047 (Parry s.n.), but

it is also consistent, although less detailed, with the label of the current lectotype. Therefore, it is plausible, but not certain, that US 1339132 was an isosyntype when Darlington designated it as the lectotype for *M. involucrata*, and it cannot be determined with confidence that this lectotypification was invalid.

Representative specimens (see Fig. 2 for map of localities). MEXICO. BAJA CALIFORNIA. **Mun. Ensenada**: Just E of Hwy 5 near ruins of ‘Casa Piedra’ about 10 miles S of Playa la Costilla, 30.1468, –114.6405, 50 m, 4 Mar 2017, *Makings 5017* (ASU 299901). **Mun. Mexicali**: 0.9 mile S of Highway 2 along road to Guadalupe Canyon, 32.5596, –115.7897, 40 m, 16 Mar 1995, *Hodgson 8884* (SD 145044); Sierra las Pintas: 0.2 miles W of Hwy 5 and 3.1 miles S of road to Ejido J. Saldaña, 31.8167, –115.1500, 50 m, 4 Mar 1988, *Prigge 7896* (LA 104828); 4.0 mi WNW of San Felipe at small hills N of main dirt road to Santa Clara, 31.0467, –114.9083, 70 m, 4 Mar 1988, *Prigge 7933* (LA 104798); Below Cantu Palms on E base of Sierra de Juarez, ca. 15 mi S of jct of Baja California Hwy 2 and Guadalupe Canyon Road, 32.3517*, –115.8276*, 100* m, 22 Mar 1986, *Thorne 61630* (LA 103806, MEXU 720847, RSA 350002); Vicinity of Santa Isabel Canyon: E side of the Sierra Juarez and W of Laguna Salada, 31.9078, –115.6744, 350 m, 16 Feb 1998, *Vinton 210* (SD 145444). SONORA. **Mun. San Luis Río Colorado**: Desierto de Altar, Sierra del Rosario, arroyo on W side of the range about 20 miles (by air) E-SE of San Luis Río Colorado, 32.0917, –114.2083, 110 m, 24 Mar 2001, *Fishbein 4520* (WS 356537). USA. ARIZONA. **Coconino Co.**: Colorado River, mile 209, Granite Park, 35.9667, –113.3167, 450* m, Apr 1975, *Theroux 1213* (ARIZ 198349). **La Paz Co.**: Highway 95 N of La Paz/Yuma county line, 33.0483, –114.2910, 254 m, 8 Mar 2008, *Brokaw 396* (WS 375826); S edge of Little Harquahala Mtns, vic. Martin Peak & Harquahala Mine, 33.6655*, –113.5909*, 600 m, 29 Mar 1992, *Christy 1088* (ASU 197786); Off Arizona Highway 95, 17.6 miles N of its junction with Highway 62 in Parker, 0.8 miles E of Highway 95, 34.2917, –114.0866, 140 m, 10 May 2003, *Hufford 4065* (WS 361990). **Maricopa Co.**: Lake Pleasant Regional Park, Bridge Canyon, 33.8776*, –112.2770*, 480* m, 1 Mar 1973, *Lehto 17520* (ASU 49387); 1 mi due S of McDowell Pk., 33.6593*, –111.8180*, 700* m, 29 Apr 1975, *R. Smith s.n.* (ASU 75802). **Mohave Co.**: Lake Havasu State Park near Highway 95, 34.3332, –114.1346, 187 m, 8 Mar 2008, *Brokaw 392* (WS 375822); Artillery Pk. Sec. 35 NW, Twp. 12N, Rge. 13W, 34.3406, –113.5854, 670 m, 13 Mar 1979, *Butterwick 4362* (ASU 113255). **Pima Co.**: Valley E of Growler Mountains, about 7 mi (air) W of Ajo, 32.3778, –112.9780, 470 m, 27 Feb 2005, *Fishbein 5491* (MISSA 34714); Foothills of the Growler Mountains, 32.04167, –112.87472, 400 m, 16 Apr 1952, *Parker 7970* (WS 217049). **Pinal Co.**: Santan Mountains Regional Park, SW corner of the park,

33.1207*, -111.6784*, 530 m, 2 Feb 2001, *Damrel 1611B* (DES 46819). **Yavapai Co.:** US Highway 93, 3.5 miles N of Santa Maria River, 34.3500, -113.2833, 900 m, 26 Mar 1960, *Thompson 3037* (LA 36881). **Yuma Co.:** Palm Canyon, Kofa Mountains, 33.3585*, -114.1065*, 655* m, 15 Apr 1979, *Hodson s.n.* (WS 347775); Palm Canyon in W side of Kofa Mountains, 33.3600, -114.1133, 605 m, 21 Mar 2003, *Hufford 4000* (WS 361961); Near Dome, 32.7459*, -114.3613*, 100* m, 10 Mar 1928, *Peebles 5049* (LA s.n.). **CALIFORNIA. Imperial Co.:** 4.5 mi s Palo Verde settlement; northeastern base of the Palo Verde Mts, 33.3577*, -114.7228*, 90 m, 18 Feb 1958, *Bacigalupi 6169* (JEPS 22541, LA 84435); Along Picacho Road, 5 miles N of its junction with old U.S. Highway 80 and 0.2 miles N of the All-American Canal, 32.7978*, -114.6172*, 60 m, 20 Mar 1962, *Bacigalupi 8315* (JEPS 89280, LA 84464); On road to Ferguson Lake, 32.9281, -114.5158, 120* m, 1 Mar 1998, *Bell 305* (SD 144576); Orocopia Mountains: 2 miles W of Clemens Well, 33.5167, -115.7000, 230 m, 21 Feb 1988, *Prigge 7847* (LA 104784, UC 1546719); W of Chocolate Mountains: ca. 1 mile E of Coachella Canal and W of Melson Well, 33.2203*, -115.3942*, 50* m, 29 Apr 1978, *Thorne 52141* (LA s.n., RSA 336271). **Inyo Co.:** Shadscale Spring-Johnson Canyon, Panamint Range, 36.0908*, -117.0078*, 1300 m, 22 May 1940, *Jepson 19648* (JEPS 41155, LA 106691); Along 4WD road 1 mile E of Baxter Mine, 36.1041, -116.2358, 920 m, 11 Apr 2015, *McCoy 15041* (IDS 66560). **Kern Co.:** W of Highway 14 near aqueduct, 35.1880, -118.1195, 953 m, 3 May 2006, *Brokaw 194* (WS 375572); Southern Sierra Nevada: Pinosa Ridge, 35.2171*, -118.0845*, 1000 m, 10 May 1957, *Twisselmann 3517* (LA 31123). **Riverside Co.:** Joshua Tree National Park: Long Canyon, c. 2.3 mi S of the NE corner of the park, 34.0607, -116.4368, 1056 m, 26 Apr 2006, *Andre 6760* (UCR 175179); Near Cottonwood Spring Road N of Interstate 10, 33.6766, -115.8120, 535 m, 9 Mar 2008, *Brokaw 400* (WS 375830); Alluvial fan, S side of Little San Bernardino Mtns., 5 mi. E of Jct Dillon Rd. and Fargo Cyn Rd., 33.7570*, -116.0287*, 800 m, 18 Feb 1988, *Charlton 1148* (LA 165817); Along Palen Pass Road, near Palen Pass, between Granite and Palen Mountains, 33.9139, -115.0772, 402 m, 10 Feb 2005, *DeGroot 4622* (RSA 709877); E side of Whitewater Canyon along Whitewater River, E of Whitewater Canyon Road, about 2 mi N of Route I-10, 33.9508, -116.6389, 549 m, 29 Apr 2001, *Hill 33541* (UCR 123754); Corn Springs, Chuckwalla Mountains, 33.6167, -115.3167, 500 m, 20 Mar 1960, *Thompson 3023* (LA 36879); Bajada beneath Mecca Hills, just E of Coachella Canal (Thermal Canyon and adj.), 33.6500, -116.0833, 24-49 m, 14 Apr 2005, *White 10892* (RSA 708489). **San Bernardino Co.:** Mojave Desert; Bagdad-Chase Mine (Bullion Mts.), 34.6297*, -116.1646*, 730* m, 25 Mar 1939, *Beal 755* (JEPS 41138, LA 106632, SD 29285); 1/2 mi. above mouth of Whitewater River Canyon, San

Bernardino Mtns, 33.9298*, -116.6420*, 600 m, 3 April 1957, *Benson 15715* (LA 105769, POM 302281); Alvord Mine; About 6 miles NE of Manix, 35.0677, -116.6286, 696 m, 26 Mar 2008, *Gross 3007* (RSA 732574, UCR 204306); Along Highway 95, 21.6 miles S of the Nevada border, 34.9576*, -114.5883*, 150* m, 10 May 2003, *Hufford 4066* (WS 361918); 14 air miles ENE of Ludlow, 17.8 miles W of Kelbaker Road on Powerline Rd, 34.8333, -115.9333, 640 m, 22 Apr 1978, *Prigge 2624* (LA 99573, RSA 278656). **San Diego Co.:** Anza-Borrego Park, N of the Volcanic Hills in Jojoba Wash about 0.1 mile W of Hwy S2, 32.8130, -116.1424, 367 m, 30 Mar 2005, *Barth 387* (SD 176683); Anza-Borrego Park, approx. 5.0 miles WSW of Imperial Co. Hwy 86 at the Grave Wash overpass bridge, 300 ft. W of county line/park boundary, 33.3056, -116.0865, 167 m, 27 Apr 2005, *Hendrickson 572* (SD 173879). Note: LA, MEXU, and RSA specimens of *Thorne 61630* are mixed collections of *M. hirsutissima* sensu stricto and *M. involucrata*.

Mentzelia nesiot (I.M.Johnst.) Brokaw & J.J.Schenk, stat. et comb. nov. *Mentzelia hirsutissima* S.Watson var. *nesiot* I.M.Johnst., Univ. Calif. Publ. Bot. 71: 443. 1922. —Type: Mexico, Baja California: Mun. Ensenada, 28 Mar 1897, *J. S. Brandegee s.n.* (holotype UC 138558!). Figs. 7, 8.

Annual herbs, (3-)8-25(-35) cm tall; taprooted. Shoots densely pubescent throughout (except as noted) with both needle-like trichomes with erect barbs and glochidiate trichomes with recurved barbs arranged in whorls. Stem erect; axillary branches ascending, straight to curved upward; stem epidermis light tan to salmon-colored, moderately pubescent. Leaves alternate, pubescent; basal rosette leaves 0.5-6.3 × 0.3-1.4 cm, oblanceolate, sessile or appearing petiolate due to narrowed lamina at base, often not persisting to maturity, margins entire to shallowly lobed, intersinus width 2.0-9.1 mm, lobes 0.9-5.0 × 0.7-2.5 mm with rounded apices; lower cauline leaves 3.1-6.7 × 0.9-2.5 cm, lanceolate, appearing petiolate to clasping, margins 5-14-lobed, intersinus width 4.2-11.0 mm, lobes 4.5-17.1 × 1.8-6.1 mm with rounded to acute apices; upper cauline leaves 2.3-4.6 × 0.9-2.9 cm, ovate to lanceolate, base often cordate-clasping, margins 7-12-lobed, intersinus width 3.5-10.7 mm, lobes 3.9-19.2 × 1.2-5.3 mm with rounded to acute apices. Inflorescence cymose, bract adnate to inferior ovary 13.1-20.9 × 5.5-10.2 mm, ovate, margins 5-9-lobed, green, not concealing pedicel or ovary. Flowers epigynous; bearing a hypanthium at the distal end of the ovary on which the calyx, corolla, and androecium are inserted. Calyx basally connate, five lanceolate lobes, lobes 9.7-15.8 × 1.9-3.1 mm, apices acute to attenuate, margins entire, pubescent; trichomes like those of leaves. Petals five, distinct, 13.5-24.1 × 11.9-22.1 mm, obovate, cream-colored to yellow (rarely orange) with veins more

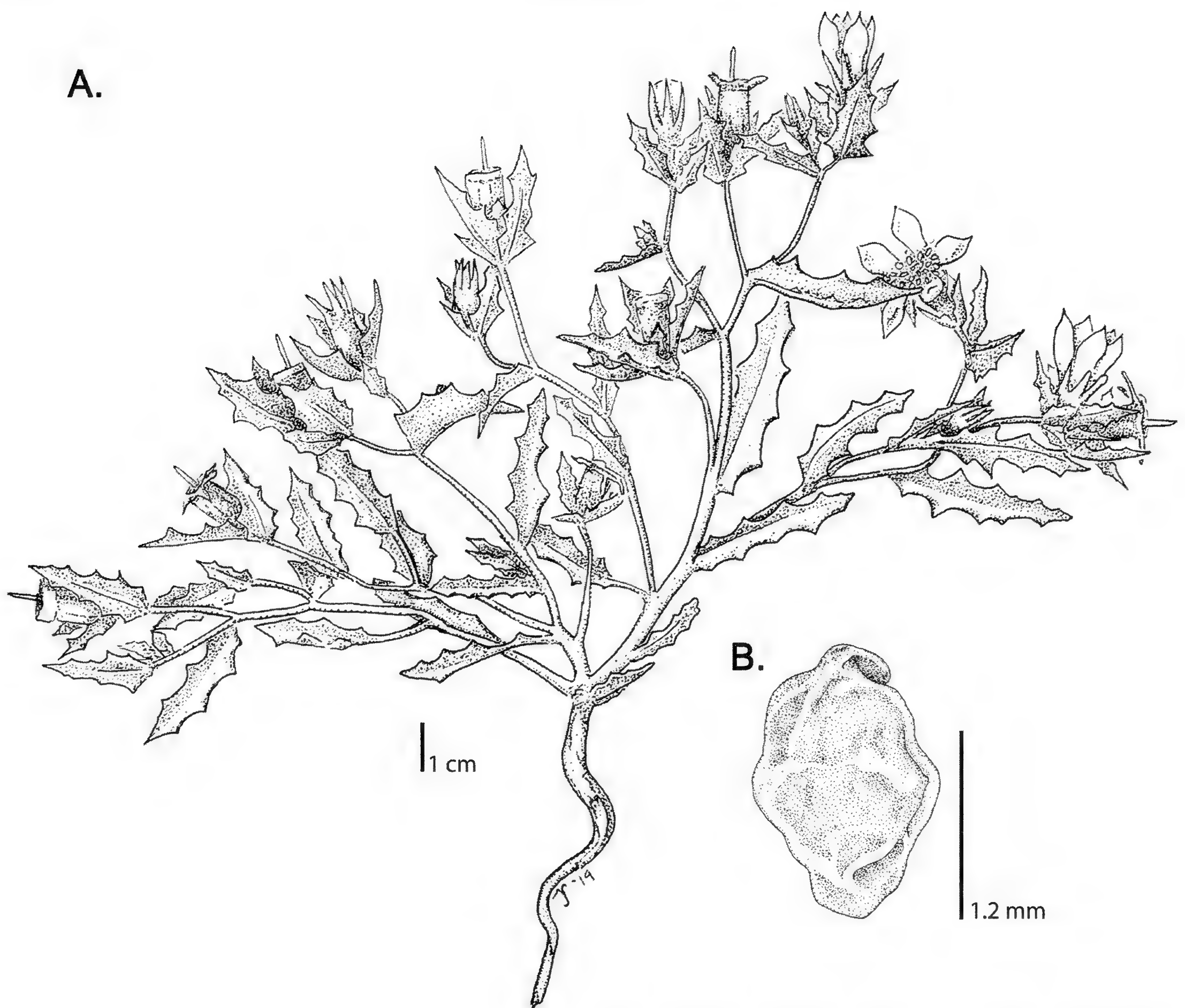


FIG. 8. Illustration of *Mentzelia nesiotens* based on *Boyd 3309* (RSA 519782). A. Habit of *M. nesiotens* and B. seed based on scanning electron micrograph from *Hufford 2680* (WS 358598).

deeply pigmented, glabrous, apex mucronate. Androecium cream-colored; stamens ca. 10–30, ca. 5.0–11.5 mm; filaments \pm monomorphic, linear, most or all distally 2-lobed with orange lateral band inferior to distal lobes, lobes to 0.3 mm; anther stalk usually longer than filament lobes, glabrous; anther epidermis papillate. Gynoecium 3-carpellate; ovaries inferior, placentae 3, parietal; styles 7.5–12.9 mm long, terete, glabrous; stigmas three, lobes appressed, papillate. Fruit a capsule, 11.3–20.9 \times 4.7–7.5 mm, cylindrical, tapering towards the base with prominent longitudinal ribs, dehiscent septically by three valves, pubescent; trichomes like those of leaves. Seeds in two series, ca. 1.8–2.0 \times 1.2–1.5 mm, widest at middle, constricted proximal to distal 1/3, flattened, rugose; seed coat pale grey; seed coat cells 20–30 μ m wide with deeply wavy anticlinal cell walls. Chromosome number not determined.

Mentzelia nesiotens is the most southern and most narrowly distributed of the species in this treatment. Populations occur primarily on rocky or sandy soils

in arroyos, canyons, and mountain slopes in the western half of Mulegé Municipality in Baja California Sur and the adjacent Pacific islands of Cedros, San Benitos, and Natividad, at 0–600 m elevation. Rebman et al. (2016) listed *M. hirsutissima* var. *nesiotens* with a California Floristic Province (CFP) status of 1B, 5: plants that are rare and endangered in CFP Baja and elsewhere, endemic to CFP. *Mentzelia nesiotens* begins flowering in mid-February and continues through late April. Plants bearing both flowers and ripe capsules with mature seeds are most common in mid-March to mid-April. By early May, most plants have senesced and disintegrated. The distribution of *M. nesiotens* does not overlap with those of any other species in *Mentzelia* section *Bicuspidaria*.

Results from molecular phylogenetic analyses (Brokaw et al. 2020) indicate that *M. involucreata* is more closely related to *M. hirsutissima* sensu stricto than is *M. nesiotens*. As demonstrated above, *M. nesiotens* is geographically, morphologically, and eco-

logically distinct from *M. hirsutissima* sensu stricto, justifying its recognition at the specific rank. However, our circumscription of *M. nesiot*es is substantially different from Johnston's (1922) protologue for *M. hirsutissima* var. *nesiot*es. In his key to *Bicuspidaria*, Johnston (1922) distinguished *M. hirsutissima* var. *nesiot*es from *M. hirsutissima* var. *hirsutissima* by its orange corolla and wider, more coarsely toothed leaves, and Johnston proposed that *M. hirsutissima* var. *nesiot*es only inhabited the Pacific islands of Cedros, San Benitos, and Natividad. Here, we acknowledge that leaf characters, including leaf intersinus width/length ratios, partially distinguish these species, but orange petals are a rare character state in both *M. nesiot*es and *M. hirsutissima* that occurred more often among *M. hirsutissima* specimens than *M. nesiot*es specimens examined in this study. Furthermore, most populations of *M. nesiot*es occur in the peninsular Vizcaíno Desert, rather than the Pacific islands, despite the meaning of the 'nesiot'es' epithet that suggests they occur on islands.

Representative specimens (see Fig. 2 for map of localities). MEXICO. BAJA CALIFORNIA. **Mun. Ensenada**: Isla Cedros, 28.1833*, -115.2000*, 300* m, 14 Mar 1939, *Elmore A5* (RSA 445122); Cedros Island, local near mouth of copper-mine canyon (near lighthouse), 1 mile from N end, 28.3614, -115.2053, 30 m, 27 Mar 1973, *Moran 20321* (SD 87010); Cedros Island, S slope of southern pine ridge N of Canyon Grande, 28.1833, -115.2000, 300 m, 30 Mar 1978, *Moran 25402* (SD 99671); Cedros Island, N end of island N of fishermen's village at lower end of Canada de la Mina, 28.3472, -115.2195, 25 m, 31 Mar 1988, *Thorne 63128* (RSA 496399). BAJA CALIFORNIA SUR. **Mun. Mulegé**: Vizcaíno Desert, plains S of Laguna Ojo de Liebre along road to Bahia Tortugas, 34 mi W of Vizcaíno Jtn, 27.5099*, -114.3080*, 240* m, 20 Apr 1989, *Boyd et al. 3291* (RSA 500906); Vizcaíno Desert, NE base of Sierra El Placer, 18.5 mi E of Rancho San Jose Del Castro on main road to Bahia Tortugas, 27.4746*, -113.7203*, 350 m, 21 Apr 1989, *Boyd 3309* (RSA 519782); Vizcaíno Peninsula S of Laguna Ojo de Liebre, at the

eastern base of the "Sierra de Vizcaíno" in a portion of the range known as Sierra Campo Nuevo, at the end of a small track winding 6.1 miles W of the road to Bahia Asunción, 3.6 miles S of the junction with the road to Bahia Tortugas, 27.3490, -114.1666, 240* m, 30 Apr 1993, *Boyd 8088* (RSA 576905); E base of Sierra de Placeros, 40 km SE of San Jose de Castro, 27.2500*, -114.2000*, 366 m, 24 Mar 1984, *Breedlove 60870* (CAS 703282, MEXU 484467); 35 km SE of Bahia Tortugas, Pacific slope of Sierra de Placeros, 27.6028*, -114.7749*, 460 m, 7 Mar 1985, *Breedlove 62325* (CAS 728337, MEXU 48474, RSA 497110); Steep W facing Canyon of the Sierra de Placeros above Puerto Nuevo between Bahia Asunción and Bahia Tortugas, 27.4899, -114.5484, 300* m, 8 Mar 1985, *Breedlove 62415* (CAS 728254, MEXU 484730); Vizcaíno Desert, eastern bajada of Sierra Calvario, Systema de Sierra Vizcaíno, 27.4035*, -114.1769*, 200* m, 10-15 Mar 1947, *Gentry 7381* (DS 323502, RSA 443118, SD 42871); Vizcaíno Desert, Cerro Tordillo and vicinity, Systema de Sierra Vizcaíno, 27.3357*, -114.3457*, 240* m, 12-13 Mar 1947, *Gentry 7418* (DS 323528, RSA 445119); Vizcaíno Desert, eastern bajada of Sierra Calvario, Systema de Sierra Vizcaíno, 27.4035*, -114.1769*, 200* m, 10-15 Mar 1947, *Gentry 7473* (RSA 445120); Arroyo 5 to 7 miles E of Los Picachos de Santa Clara, 27.2608*, -113.6431*, 190* m, 23 Mar 1947, *Gentry 7600* (DS 323803, RSA 445117); Vizcaíno Desert, Arroyo de Tecolote near lava flows, 27.4128*, -113.7293*, 80* m, 19 Nov 1947, *Gentry 7829* (DS 323560, RSA 445121); Vizcaíno Desert, 80.6 km W of Highway 1 in Vizcaíno Junction and 7.6 km NW of junction with road to Bahia Asunción along road to Bahia Tortugas, 27.4719*, -114.1013*, 30 m, 17 Mar 1998, *Hufford 2680* (WS 358598); N slope of SE peak, Picachos de Santa Clara, 27.1167, -113.6167, 600 m, 3 Feb 1973, *Moran 19706* (SD 92316); Arroyo 8 road miles NW of Asunción, 27.2148, -114.3502, 70 m, 4 Feb 1973, *Moran 19767* (SD 92312); Arroyo Calvario 6 miles N of San Andres, 27.3166, -114.4344, 130 m, 10 Feb 1973, *Moran 20008* (SD 92284).

KEY TO MENTZELIA SECTION BICUSPIDARIA

1. 1-3(-5) outermost stamens staminodial and petaloid (flowers appearing to have 6-8(-10) petals); stamen filaments distally unlobed; petals lanceolate to oblanceolate, 6-12 mm, apices acute; styles 5-6.5 mm
..... *M. reflexa*
- 1'. Stamens all fertile, none petaloid (flowers appearing to have 5 petals); stamen filaments all or most distally 2-lobed; petals obovate, 10-65 mm, apices mucronate; styles 6-30 mm
2. Bracts mostly concealing pedicels and capsules, white with green margins *M. involucrata*
- 2'. Bracts not mostly concealing pedicels or capsules, green (excluding the midvein, which can be white)
3. Stamen filament lobes 0.6-2.5 mm, anther stalk usually shorter; seeds constricted and grooved at middle *M. tricuspis*
- 3'. Stamen filament lobes 0.1-0.7 mm, anther stalk equal or longer; seeds widest at middle, not grooved
4. Petioles present in distalmost cauline leaves, blade base not cordate-clasping; proximal capsules nodding, distal erect; central Mojave Desert *M. tridentata*
- 4'. Petioles absent in cauline leaves, blade base often cordate-clasping; capsules erect; southwestern edge of the Sonoran Desert
5. Ratio of intersinus width/leaf length in lower cauline leaves < 0.1(0.15); capsule length (16.7-)18.5-31.4 mm from pedicel to base of calyx lobes; calyx lobes (12.6-)14.3-25.2 mm

- long; Mexicali, Tecate, and Ensenada Municipalities (excluding Pacific islands), Baja California and Tiburón Island, Sonora, Mexico; San Diego and Imperial Counties, California, USA; north of 28.5°N latitude *M. hirsutissima*
- 5'. Ratio of intersinus width/leaf length in lower cauline leaves > 0.1(0.08); capsule length 11.3–18.5(–20.9) mm from pedicel to base of calyx lobes; calyx lobes 4.5–14.3(–17.1) mm long; Mulegé Municipality, Baja California Sur and Cedros, San Benitos, and Natividad Islands, Baja California, Mexico; south of 28.5°N latitude. *M. nesiotés*

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Environmental niche equivalency versus conservatism:

A LIKELY INTERSECTIONAL HYBRID IN *VACCINIUM* (ERICACEAE) ON SAN BRUNO MOUNTAIN, SAN MATEO COUNTY, CALIFORNIA

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ABSTRACT

A population of *Vaccinium* from San Bruno Mountain in San Mateo County, California has been confused with *V. cespitosum* Michx. (*V. sect. Myrtillus*), a species documented from the mountain, since its initial collection in 1961. These plants resemble *V. cespitosum* and other species in *V. sect. Myrtillus* in several characters, but differ most notably in their evergreen habit and well developed calyx lobes. The latter characters are shared by *V. ovatum* Pursh (*V. sect. Pyxothamnus*), the only other species of *Vaccinium* known from San Bruno Mountain, suggesting that the population is a hybrid between *V. cespitosum* and *V. ovatum*. We used data from gross morphology, leaf anatomy, and DNA sequences from the nuclear ribosomal internal transcribed spacer (ITS) and plastid *matK* and *ndhF* regions to test the hybrid status of this population. Hybrid status is supported by: 1) a plastid sequence profile identical to that of *V. cespitosum* from San Bruno Mountain and different from the profiles of all other *Vaccinium* samples in the study, 2) an ITS profile that differs from *V. cespitosum* from the mountain by only two polymorphic sites, and 3) the possession of gross morphological and anatomical characters that are either shared with one putative parent or the other, or are intermediate between them. Although ITS variation was non-additive and closely matched one of the putative parents, uniparental inheritance has been observed in other hybrids. Two novel gross morphological characters were also observed in the hybrid. The study documents a case of likely intersectional hybridization within *Vaccinium*, only rarely observed under natural conditions. *Vaccinium cespitosum* may have been outcompeted by the hybrid to the point of its extirpation from San Bruno Mountain. The population is here newly described as the nothospecies *Vaccinium* \times *brunoense* P.W.Fritsch, occurring in an area of only ca. 200 m² with the number of individuals remaining uncertain because of clonal growth.

Key Words: California, Ericaceae, hybrid, leaf anatomy, phylogenetics, San Bruno Mountain, San Mateo County, *Vaccinium*.

San Bruno Mountain, located in northern San Mateo County, California, harbors a unique and rich flora, including a number of rare, endangered, and endemic plants (McClintock et al. 1968, 1990; D. D. Allshouse, unpublished data). The uniqueness of the flora is largely due to its rugged and irregular topography (elevation from near sea level to 400 m) and diversity of weather patterns, creating many microclimates and associated vegetation types. Perhaps most notable is the presence of fog drip in many areas due to marine influence, resulting in appreciable water availability throughout much of the year on otherwise dry slopes, unlike the surrounding predominant pattern of summer drought. The area's unique flora is also due to the status of the mountain as the largest remnant of the Franciscan Landscape, a floristic region of high endemism that has been virtually eliminated elsewhere (Gaar and Miller 2006).

One of the families of plants with rare or unusual taxa on San Bruno Mountain is the Ericaceae, with four genera and nine species. *Arctostaphylos* Adans. is especially notable with five species, two of which are endemic to the mountain and one of which occurs only on one other mountain. Another species (*A. uva-ursi* (L.) Spreng.) is represented by three distinct variants sometimes recognized as forms (McClintock et al. 1990; Kauffmann et al. 2015), two of which are endemic. Two widespread species of *Vaccinium* L. are also documented, *V. cespitosum* Michx. (dwarf bilberry) and *V. ovatum* Pursh (California huckleberry). *Vaccinium ovatum* is common on the mountain, forming a shorter and more compact shrub than is typical for the species, whereas *V. cespitosum* is known only from "Kamchatka Point," a northward-extending ridge spur on the northeast side of the mountain (McClintock et al. 1968, 1990; *V. cespitosum* is referred to as the synonym *V. arbuscula*

(A.Gray) Merriam in McClintock et al. 1968) and as such represents the southernmost non-Sierra Nevada locality of this species in California.

During field work on San Bruno Mountain beginning in 2012, authors Fritsch and Allshouse studied the plants at Kamchatka Point identified by McClintock et al. (1990) as *V. cespitosum*, finding that several characters deviated substantially from those described for *V. cespitosum* from the U.S. They differ most notably in their evergreen habit and flowers with five well developed calyx lobes (versus deciduous and a flat or at most undulate-margined calyx). In comparing photographs and herbarium collections made during this field work to the historical collections at the California Academy of Sciences (CAS) of *Vaccinium* from San Bruno Mountain (made from 1961 to 1988), the first author discovered that some CAS collections identified as *V. cespitosum* appeared to harbor two superficially similar, but distinct entities mixed on the sheets, one with thin leaves and one with thicker leaves. All the plants have perennating buds with two partially fused scales, consistent with *V. cespitosum* and diagnostic for *V. sect. Myrtilus* Dumort. among *Vaccinium* in the western U.S. (Vander Kloet 1988, 1996). Moreover, the thinner-leaved plants of these collections possess characters that are all consistent with those of *V. cespitosum* from the northwestern U.S., e.g., weakly angled branchlets, relatively thin leaves (presumably deciduous, as in all *V. cespitosum* of the U.S. and Canada), green (not glaucous) leaf blades abaxially, serrulate leaf blade margins, 1-flowered inflorescences on branchlets of the current season, ebracteolate pedicels that are not articulated with the flowers, a flat or at most undulate calyx margin, glabrous stamen filaments, and 5-locular ovaries (Vander Kloet 1988; Wallace 2012; P. W. Fritsch, personal observation). In contrast, the thicker-leaved (evergreen) plants were found to differ from *V. cespitosum* by a substantial number of characters (Table 1). Other characters of the evergreen plants not only fail to match those of *V. cespitosum*, but are unknown in any North American or north-temperate member of *V. sect. Myrtilus* (Vander Kloet 1988, 1996; Vander Kloet and Dickinson 2009; P. W. Fritsch, personal observation; Table 1). Repeated searches on San Bruno Mountain have failed to yield either any of the thinner-leaved plants or any populations of the thicker-leaved plants besides the one at Kamchatka Point.

We considered the unusual features of the San Bruno Mountain population of *Vaccinium* to warrant further investigation of its origin and taxonomic status. Most plausibly it either originated through hybridization between *Vaccinium* species that occur on San Bruno Mountain, or else is an undescribed species. Several features of the population lend credence to the hybridization hypothesis. Many species of *Vaccinium* are known to hybridize both naturally and in artificial crosses (Camp 1942; Lyrene et al. 2003; Brevis et al. 2008). The only other

Vaccinium species known from the mountain besides *V. cespitosum* is *V. ovatum*, the sole north-temperate member of *V. sect. Pyxothamnus* (Nutt.) Sleumer. That section differs from the north-temperate members of *V. sect. Myrtilus* in a substantial number of characters (Stevens 1969; Vander Kloet 2009; Vander Kloet and Dickinson 2009; Table 1). In the leaf persistence, bracteole, calyx lobe, and stamen filament characters, the unusual evergreen population on San Bruno Mountain (henceforth “evergreen ‘*V. cespitosum*’”) resembles *V. ovatum*; thus, for several characters the population has either one or the other character state corresponding to the respective sections, suggesting a hybrid origin (Rieseberg et al. 1993). Furthermore, fertility is observed as low in the population of evergreen “*V. cespitosum*,” with fruit rarely developing, a trait common in many *Vaccinium* hybrids (Buerkle et al. 2000). In addition, at Kamchatka Point *V. ovatum* is common, whereas *V. cespitosum* is rare and apparently now extirpated, consistent with the idea that a common congener can demographically swamp and genetically assimilate a rare species through hybridization and lead to the extinction of rare species or extirpation of rare populations (Levin et al. 1996). Finally, hybridization would be consistent with the presence of only a single population of these plants because even though there are many above-ground stems in the population, extensive rhizomes are evident, and the entire population could be a clonal single F1 individual.

Nonetheless, other features of the population support the hypothesis that it represents an undescribed species. Although homoploid species crosses within sections of *Vaccinium* usually result in fertile hybrids, those among sections are at best of low fertility unless the parents are both tetraploid (Vander Kloet 1988; Lyrene et al. 2003; Chavez and Lyrene 2010; Lyrene and Olmstead 2012). The two species on San Bruno Mountain are in different sections, and both are diploids (Vander Kloet 1988), suggesting that the population of evergreen “*V. cespitosum*” is not a hybrid. However, vigorous *Vaccinium* hybrids of diploid parents have been produced (Ehlenfeldt and Polashock 2014) and there is evidence for the rare occurrence of natural intersectional hybrids, including one case of diploid parentage (Ritchie 1955a; Powell and Kron 2002). Moreover, San Bruno Mountain is known for harboring rare plant species that were once more common on the San Francisco Peninsula, but are now extirpated due to human activities (McClintock et al. 1968), and thus an undescribed rare species would be consistent with the general pattern of plant rarity and endemism documented for the mountain. Finally, the evergreen “*V. cespitosum*” plants bear particular resemblance to the Hawaiian species of *Vaccinium*. These species (*V. calycinum* Sm., *V. dentatum* Sm., and *V. reticulatum* Sm.; Vander Kloet 1999) are included within *V. sect. Myrtilus* based on multiple characters (e.g., their perennating buds with

TABLE 1. COMPARISON OF MACROMORPHOLOGICAL CHARACTERS AMONG EVERGREEN “*VACCINIUM CESPITOSUM*,” *V. CESPITOSUM*, THE NORTH-TEMPERATE MEMBERS OF *V. SECT. MYRTILLUS*, AND *V. OVATUM* OF *V. SECT. PYXOTHAMNUS*. N/A signifies not applicable. Characters in boldface type are differences between *V. sect. Pyxothamnus* and the north-temperate members of *V. sect. Myrtillus*.

Character	Evergreen “ <i>V. cespitosum</i> ”	<i>V. cespitosum</i>	<i>V. sect. Myrtillus</i>	<i>V. ovatum</i>
Plants	Evergreen	Deciduous (except some in Mexico)	Deciduous (except some in Mexico)	Evergreen
Branchlet width (mm)	0.4–0.6	0.5–1.2	N/A	0.8–2.5
Branchlet trichomes (mm)	≤ 0.3	≤ 0.15	N/A	≤ ca. 6
Perennating buds toward the shoot apices	Monomorphic	Monomorphic	Monomorphic	Dimorphic
Perennating bud scales	2, partially fused	2, partially fused	2, partially fused	> 2, distinct
Leaf blade thickness	Subcoriaceous	Membranaceous	N/A	Coriaceous
Leaf blade tertiary veins abaxially	Less prominent and obscure on older leaves	Relatively prominent	N/A	Obscure
Leaf blade glands abaxially	Sparse	Sparse	N/A	Dense
Glandular trichome position on leaf serrations	At ± middle of upper edge of tooth	At or near outer portion of upper edge of tooth	N/A	Ranging from ± middle of upper edge of tooth to notch of tooth
Inflorescences	1-flowered on branchlets of current season and 2- to 3-flowered fasciculate inflorescences on branchlets of previous season	1-flowered on branchlets of current season	1-flowered on branchlets of current season	Racemes on branchlets of previous season or older
Pedicels (bracteole presence)	Bracteolate	Ebracteolate	Ebracteolate	Bracteolate
Pedice length (mm)	0.3–0.9	2–6	N/A	2–8
Pedice articulation with flower	Present but faint	Absent	Absent	Present
Calyx lobes	Well developed	Flat, undulate or weakly lobed	Flat, undulate or weakly lobed	Well developed
Calyx lobe apex	Ciliate	Eciliate	Eciliate	Ciliate or eciliate
Corolla shape	Broadly urceolate	Cylindric-urceolate	N/A	Broadly urceolate
Stamen filaments	Pubescent	Glabrous	Glabrous	Pubescent
Anther spur length (mm)	0.1–0.8	Ca. 1.5	N/A (but present)	Absent or < 0.1

TABLE 2. NEWLY SEQUENCED SAMPLES FOR THIS STUDY WITH VOUCHER INFORMATION AND GENBANK NUMBERS. Asterisks (*) indicate samples obtained from an herbarium specimen.

Species	Collection (Herbarium)	Locality	GenBank No.		
			ITS region	<i>matK</i>	<i>ndhF</i>
<i>V. cespitosum</i> Michx.	<i>Fritsch 1968</i> (CAS)	Point Reyes, CA, USA	MT603495	MT593008	MT593003
<i>V. cespitosum</i>	<i>McClintock & Menzies s.n.</i> (CAS)*	San Bruno Mt., CA, USA	MT603496	MT593010	MT593005
<i>V. cespitosum</i>	<i>Thomas 10264</i> (DS)*	San Bruno Mt., CA, USA	MT603497	MT593009	MT593004
Evergreen “ <i>V. cespitosum</i> ”	<i>Fritsch 1967</i> (CAS)	San Bruno Mt., CA, USA	MT603498	MT593011	MT593006
<i>V. ovatum</i> Pursh	<i>Fritsch 1939</i> (CAS)	San Bruno Mt., CA, USA	MT603499	MT593012	MT593007

two partially fused scales, one-flowered inflorescences, spurred anthers, and 5-locular ovaries), and molecular data have supported this placement (Powell and Kron 2002). They differ from all other species of *V. sect. Myrtilus* by, most notably, their (often) consistent evergreen habit, well developed calyx lobes, and (often) pubescent stamen filaments, all of which are present in evergreen “*V. cespitosum*.” This and the fact that North America is an important source area for the Hawaiian flora (Baldwin and Wagner 2010) suggested to us that evergreen “*V. cespitosum*” may be a distinct species in *V. sect. Myrtilus* and closely related to the Hawaiian species.

Data from DNA sequences have been used effectively to test the hybrid status of populations by searching for patterns of additivity of polymorphic sites in nuclear ribosomal (nr) DNA sequence profiles, as well as identical plastid haplotypes between the putative hybrid and one of the parents (e.g., Rieseberg et al. 1993; Baldwin et al. 1995; Aguilar and Feliner 2003; Lu et al. 2010). Here we use DNA sequence data in combination with macro-morphology and leaf anatomy to test whether evergreen “*V. cespitosum*” is a naturally occurring hybrid between *V. cespitosum* and *V. ovatum* or an undescribed species. In concluding that the preponderance of evidence favors hybridization, we describe the plants as a nothospecies and provide a detailed description, an illustration, and a list of specimens examined.

MATERIALS AND METHODS

DNA Sequence Data

Five samples were used to generate new DNA sequence data: one from the evergreen “*V. cespitosum*” population, one from a plant of *V. ovatum* from San Bruno Mountain, two from herbarium specimens of *V. cespitosum* from San Bruno Mountain because living plants of this species have not been relocated on the mountain, and one from a population of *V. cespitosum* from Point Reyes National Seashore near Abbotts Lagoon with which to compare the data from the *V. cespitosum* samples from San Bruno Mountain (Table 2). DNA was extracted from fresh field-collected material. A Mini-BeadBeater 16 (BioSpec Products, Inc., Bartlesville,

OK) was used to grind the tissue samples, and total genomic DNA was then extracted with the DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, CA) as per the manufacturer’s protocols. Standard PCR techniques were used to amplify all targeted regions (Dieffenbach and Dveksler 2003) except that Hot-Start-It *Taq* polymerase (Affymetrix, Santa Clara, CA) was used for amplifications. Two plastid regions, *matK* and *ndhF*, and the nr internal transcribed spacer (ITS) region (i.e., including the 5.8S coding region), were used in the analysis. These regions were used because they have been most commonly employed in molecular phylogenetic analyses of the tribe Vaccinieae (Kron et al. 2002; Tsutsumi 2011), including a study focusing on *V. sect. Myrtilus* (Powell and Kron 2002).

The regions were amplified and directly sequenced with the Sanger method by using the following primers: for the ITS region, White et al. (1990) and Swensen et al. (1998); for *matK*, external primers 1F and 1100R, 2R, or trnK-2R as based on sequence information from Johnson and Soltis (1994) and Steele and Vilgalys (1994), with internal primers 1295F and 1600R from Fritsch et al. (2011) and internal primers 650F and 1350R from Powell and Kron (2001); and for *ndhF*, primers 1F, 972R, 1036F, and 1824R from Olmstead and Sweere (1994), Alverson et al. (1999), and Bush et al. (2009), plus new primers modified specifically to match the sequence of *V. macrocarpon* Aiton (Fajardo 2013): (all 5’–3’) V1F: ATGGAACATACATATCAA-TATGC; V1036F: TTAGGAGCTACTTTAGCAC; V1824R: CCAAAGTCATTATGGAGTGATCG; and V972R: CATAATATATCCTAATTGAGAC. An additional new primer, V465R (CATTTGCTG-CAASAGGCCTT), was designed to amplify and directly sequence the *ndhF* 5’ end of the *McClintock & Menzies s.n. V. cespitosum* sample. DNA sequencing was performed as in Fritsch et al. (2015).

To assess the phylogenetic placement of evergreen “*V. cespitosum*” we incorporated the newly generated sequences into datasets constructed from a sample of ITS region, *matK*, and *ndhF* sequences included in a prior study (Powell and Kron 2002) and downloaded from GenBank. We included enough species in the datasets to sufficiently test the placement of the new samples in the clades recovered from the prior analyses. For completeness, we also included all

ITS region and complete *matK* sequences of *V. cespitosum* and *V. ovatum* available from GenBank (Hileman et al. 2001; Vander Kloet et al. 2004; Matuszak 2015). Other *matK* sequences from these species in GenBank were only partial sequences relative to our datasets. Preliminary phylogenetic analyses of single genes with these sequences yielded essentially no additional insights into the taxonomic status of evergreen “*V. cespitosum*” and were thus not included in final analyses.

The ITS and plastid datasets both included the same 28 species of *Vaccinium*, one species of *Gaylussacia* Kunth in the same tribe as *Vaccinium* (Vaccinieae), and one outgroup species of *Leucothoe* D. Don of the tribe Gaultherieae. Because of the more extensive data available for ITS data, the ITS dataset comprised 43 terminals and the plastid dataset 33 terminals. The datasets included all species of *V. sect. Myrtillus*. Sequences were edited with Geneious 11.1.5 (Biomatters Ltd., Auckland, NZ) and aligned with the MAFFT plugin (Kato and Standley 2013). The concatenated alignment was divided into biologically meaningful partitions corresponding to each of the three genic regions. MrModeltest2 version 2.3 (Nylander 2004) was implemented with PAUP* (Swofford 2002) to estimate substitution models for each partition under the Akaike Information Criterion, with the best-fitting model subsequently applied to each partition for the Bayesian analyses. We employed maximum likelihood (ML) and Bayesian inference (BI) analyses to generate phylogenetic trees as in Fritsch et al. (2015). The ML analysis was conducted with RAxML 7.2.6 (Stamatakis et al. 2008) by employing the General Time Reversible model of nucleotide substitution under the Gamma model of rate heterogeneity (GTRGAMMA) with clade support estimated with ML bootstraps (BP). The BI analysis was conducted by using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with clade support measured as posterior probability (PP) values > 0.5. We analyzed ITS region and plastid data partitions separately to assess the hybrid status of evergreen “*V. cespitosum*.”

Leaf Anatomy

To assess whether evergreen “*V. cespitosum*” shares leaf anatomical characters with deciduous *V. cespitosum*, *V. ovatum*, or Hawaiian members of *V. sect. Myrtillus*, we prepared leaf samples for anatomical examination from six plants. Fresh field-collected leaves from an individual of evergreen “*V. cespitosum*” (California, San Mateo Co., San Bruno Mountain County Park, *P. W. Fritsch* 2239 (BRIT)) and *V. ovatum* [California, San Mateo Co., San Bruno Mountain County Park, 30 June 2016, *D. Allshouse s.n.* (BRIT)] were placed in 70% ethanol and later used for the study. Other leaf samples were taken from dried herbarium specimens: *V. cespitosum* (California: San Mateo Co., San Bruno Mountain, *J.*

H. Thomas 10264 (DS), and Marin Co., Point Reyes National Seashore, *P. W. Fritsch* 1968 (CAS)); and two Hawaiian species of *V. sect. Myrtillus*, i.e., *V. calycinum* (Hawaii: Hawaii Island, 27 Miles, Volcano, *O. Degener* 34837 (BRIT)) and *V. reticulatum* (Hawaii: Maui Island, W slope of Kuiki, Haleakala, *O. Degener* 12494 (BRIT)).

Three to seven leaves from each species were placed in glass petri dishes and covered with 5% sodium hydroxide at 37°C for 3 to 7 d until all pigment was removed and the leaves were translucent. Leaves were then rinsed in water, stained with 1% Safranin O in 50% ethanol for several minutes or overnight, and dehydrated in 70% ethanol rinse (5 min). They were then rinsed in 95% ethanol, moved to a 1:1 mix of absolute ethanol/xylene, and stored in xylene. Whole, cleared leaves were mounted on 3 × 1-inch slides with Histomount™ (National Diagnostics, Atlanta, GA) and covered with a cover slip. For thin sectioning, dried leaves were fixed in 70% ethanol, dehydrated in a tertiary butyl alcohol series, and embedded in 56.5°C TissuePrep™ (Thermo Fisher Scientific, Waltham, MA). A paradermal section cut at 12-μm thickness on a conventional rotary microtome was performed for *V. ovatum* to examine the veinlet endings which tended to be obscured by densely staining cells in these thick leaves. Sections were stained in Safranin O as per standard microtechnique procedures (Johansen 1940).

Permanent slides of both cleared and thin sectioned leaves were added to the BRIT microslide collection. An Olympus (Center Valley, PA) model AX70 with model DP71 digital camera attachment was used to examine material and capture images. For each collection, the area of leaf areoles was measured from among three different leaves at a location near the middle of the lamina halfway between the margin and the midvein. Leaf images were captured at 40× magnification and area was measured and calculated with ImageJ ver. 1.52c (Schneider et al. 2012).

RESULTS

DNA Sequence Data

The ML and BI trees from both ITS and plastid data are generally consistent with those previously generated from these regions with parsimony analysis (Kron et al. 2002; Powell and Kron 2002; Tsutsumi 2011). In the ITS region analysis, overall clade support is weak (Fig. 1). Except for *V. cereum* G. Forst., the species of *V. sect. Myrtillus* form a clade (ML BP = 71, PP = 0.94), but only with the inclusion of *V. erythrocarpum* Michx., *V. hirtum* Thunb., *V. praestans* Lamb., and *V. smallii* A. Gray from other sections; all these sections share the character of perennating buds with two partially fused scales. *Vaccinium cereum* groups as sister to *V. fragile* Franch. (ML BP = 94, PP = 1.00). The evergreen “*V. cespitosum*” sample and the samples of

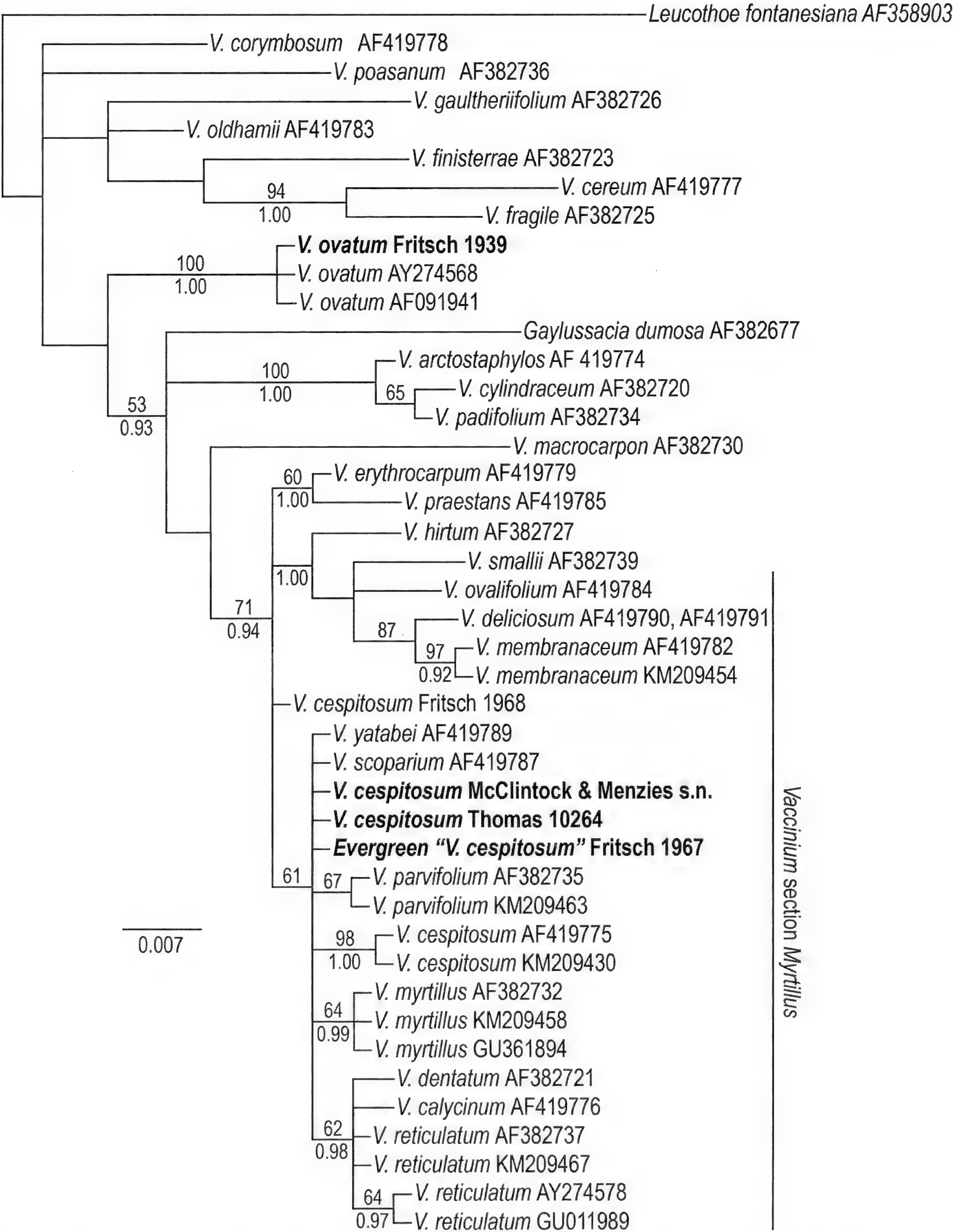


FIG. 1. Bayesian majority-rule consensus tree from a phylogenetic analysis of 28 species of *Vaccinium*, one species of *Gaylussacia*, and one outgroup species of *Leucothoe* (43 terminals) based on ITS region DNA sequence data. The dataset includes all species of *V. sect. Myrtillus* with available molecular data from prior studies, as well as the new samples from San Bruno Mountain (in boldface) *V. ovatum* (Fritsch 1939), evergreen "*V. cespitosum*" (Fritsch 1967), and two herbarium samples of *V. cespitosum* (McClintock & Menzies s.n. and Thomas 10264). GenBank accession numbers are indicated after species names. Branch support values are indicated along branches, with Bayesian posterior probabilities > 0.90 above and maximum likelihood bootstrap values > 50 (tree not shown) below. *Vaccinium cereum* groups outside of the *V. sect. Myrtillus* clade, as in Powell and Kron (2002).

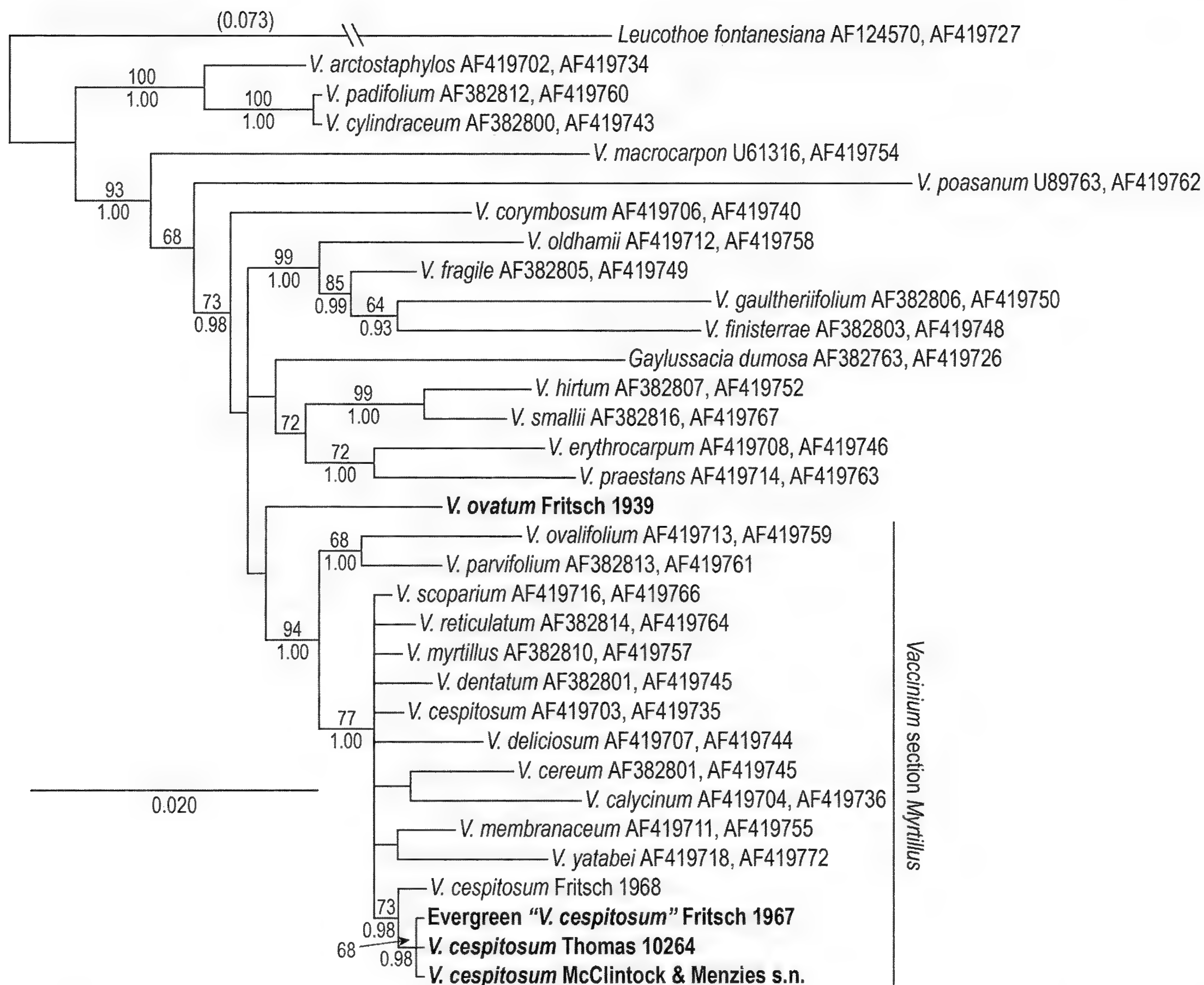


FIG. 2. Bayesian majority-rule consensus tree from a phylogenetic analysis of 28 species of *Vaccinium*, one species of *Gaylussacia*, and one outgroup species of *Leucothoe* based on plastid DNA sequence data (*matK* and *ndhF*). The dataset includes all species of *V. sect. Myrtillos* with available molecular data from a prior study, as well as the new samples from San Bruno Mountain (in boldface) *V. ovatum*, evergreen "*V. cespitosum*" (Fritsch 1967), and two herbarium samples of *V. cespitosum* (McClintock & Menzies s.n. and Thomas 10264). GenBank accession numbers are indicated after species names. Branch support values are indicated along branches, with Bayesian posterior probabilities > 0.90 above and maximum likelihood bootstrap values > 50 (tree not shown) below. The value in parentheses indicates the actual length of the outgroup branch, truncated in the figure. *Vaccinium cereum* groups within the *V. sect. Myrtillos* clade, as in Powell and Kron (2002).

V. cespitosum all group within the *V. sect. Myrtillos* clade. Species relationships are mostly unresolved within this clade or exhibit low support (ML BP < 50, PP < 0.90), although the Hawaiian species *V. calycinum*, *V. dentatum*, and *V. reticulatum* form a clade with low ML support (BP = 62), but high Bayesian support (PP = 0.98). *Vaccinium ovatum* groups outside of the *V. sect. Myrtillos* clade as sister to a large clade.

Clades recovered in the trees from the plastid analyses are more highly resolved than in the trees from the ITS analysis (Fig. 2). *Vaccinium sect. Myrtillos* forms a strongly supported clade (ML BP = 94, PP = 1.00). The evergreen "*V. cespitosum*" sample forms a clade with all newly sequenced samples of *V. cespitosum* with moderate to strong support (ML BP = 73, PP = 0.98). Moreover, both

samples of *V. cespitosum* from San Bruno Mountain form a clade with the evergreen "*V. cespitosum*" with moderate to strong support (ML BP = 68, PP = 0.98), with the sample from Point Reyes grouping as sister to this clade, and with the sample from a previous study (Powell and Kron 2002) grouping as unresolved relative to most clades in the *V. sect. Myrtillos* clade. Other species relationships are mostly unresolved within the *V. sect. Myrtillos* clade. In contrast to its placement in the ITS trees, *V. cereum* groups within the *V. sect. Myrtillos* clade. *Vaccinium ovatum* groups as sister to the *V. sect. Myrtillos* clade, with weak support (ML BP < 50, PP < 0.90).

In the ITS region dataset, only one base is polymorphic in evergreen "*V. cespitosum*" (site 36 in the aligned dataset, C + T (= Y); Table 3), and the

TABLE 3. VARIABLE NUCLEOTIDE POSITIONS IN THE ITS REGION AMONG THE SAMPLES OF *VACCINIUM CESPITOSUM*, EVERGREEN “*V. CESPITOSUM*,” AND *V. OVATUM*. Polymorphisms within samples, and nucleotides with the lowest frequency across all samples in the table per position, are indicated in boldface. The GenBank accession numbers of *Boivin* 482 (Quebec, Canada), *Vander Kloet* 313887 (locality unknown), *Vander Kloet* 231879 (British Columbia, Canada), and *YAU 018* (locality unknown) are KM209430, AF419775, AY274568, and AF091941, respectively; for GenBank and locality information of other samples, see Table 2. Dashes (-) indicate missing data.

Species	Collection	Position																		
		36	61	63	76	114	149	152	153	180	198	452	455	470	515	559	571	587	608	641
<i>V. cespitosum</i>	<i>Boivin</i> 482	C	C	G	G	A	T	G	T	A	C	C	T	A	G	A	C	A	T	T
<i>V. cespitosum</i>	<i>Vander Kloet</i> 313887	C	C	G	G	A	T	G	T	A	C	C	T	A	G	A	C	A	T	T
<i>V. cespitosum</i>	<i>Fritsch</i> 1968	Y	C	G	R	A	T	S	C	A	Y	Y	T	R	G	A	C	A	Y	C
<i>V. cespitosum</i>	<i>McClintock</i> & <i>Menzies</i> s.n.	C	C	G	G	A	T	G	C	A	Y	C	T	A	G	A	C	A	T	C
<i>V. cespitosum</i>	<i>Thomas</i> 10264	C	C	G	G	A	T	G	C	A	Y	C	T	A	G	A	C	A	T	C
Evergreen “ <i>V. cespitosum</i> ”	<i>Fritsch</i> 1967	Y	C	G	G	A	T	G	C	A	C	C	T	A	G	A	C	A	T	C
<i>V. ovatum</i>	<i>Fritsch</i> 1939	C	T	C	A	G	C	G	C	G	C	C	C	G	T	G	T	G	T	C
<i>V. ovatum</i>	<i>Vander Kloet</i> 231879	C	T	C	A	G	C	G	C	G	C	C	C	G	T	G	T	G	T	C
<i>V. ovatum</i>	<i>YAU</i> 018	C	T	C	A	G	C	G	C	G	C	C	C	G	T	G	T	G	-	-

samples of the putative parents on San Bruno Mountain—*V. cespitosum* *E. McClintock* & *A. Menzies* s.n. and *J. H. Thomas* 10264, and *V. ovatum* *P. W. Fritsch* 1939—are monomorphic for “C” at this site, i.e., there were no additive ITS region polymorphisms detected in the putative hybrid. Furthermore, there are no variable ITS region sites in which the evergreen “*V. cespitosum*” sample unambiguously and uniquely shares the same base with the *V. ovatum* sequences (Table 3). In contrast, there are 12 variable ITS sites in which the evergreen “*V. cespitosum*” sample unambiguously and uniquely shares the same base with the *V. cespitosum* samples. There are no variable ITS region sites that show a unique base in the evergreen “*V. cespitosum*” sample versus the five samples of *V. cespitosum*. Except for the polymorphic bases within samples at sites 36 and 198, the ITS sequences of the samples of evergreen “*V. cespitosum*” and *V. cespitosum* from San Bruno Mountain are identical.

In the plastid dataset, there are 30 variable sites among *V. cespitosum*, evergreen “*V. cespitosum*,” and *V. ovatum*; nine of these occur in *matK* and 21 in *ndhF* (Table 4). Of these sites, 25 show different bases between the evergreen “*V. cespitosum*” and *V. ovatum* haplotype (eight in *matK* and 17 in *ndhF*), whereas the sites are invariant among evergreen “*V. cespitosum*” and the two samples of *V. cespitosum* from San Bruno Mountain, i.e., the haplotypes are identical except for some missing values in *ndhF* (Table 4). In contrast, three sites differ between the evergreen “*V. cespitosum*” and *V. cespitosum* from Point Reyes.

Leaf Anatomy

All species of *Vaccinium* leaves studied had pinnate, camptodromous (reticulodromous) venation. The primary vein terminates at the leaf apex.

Secondary veins recurve to join other secondary veins or branch to form a reticulate network, but do not extend to the leaf margin. Higher-order venation is typically reticulate. Areoles are predominately quadrangular (Fig. 3) with free-ending veinlets of variable lengths, branching, and curvature. Areole size, the presence of bundle sheath cells around the veins (common in members of the Ericaceae; Rao and Dickison 1985), the extension of bundle sheath cells around and beyond the tracheid/vein endings, and swelling of the terminal tracheids at the ends of veinlets, often referred to as “clubbing” (Fig. 4) were generally consistent within samples, although substantial variation was observed.

The leaf veinlet areoles of *V. cespitosum* from Point Reyes are on average the largest from among all the leaves sampled for anatomical examination, followed by those of *V. cespitosum* from San Bruno Mountain, evergreen “*V. cespitosum*,” and *V. ovatum*; those from the two Hawaiian species (*V. calycinum* and *V. reticulatum*) are the smallest (Table 5, Fig. 3). The terminal veinlets of *V. cespitosum* from San Bruno Mountain, Point Reyes, and evergreen “*V. cespitosum*” are all generally oriented toward the petiole, whereas those of the Hawaiian species and *V. ovatum* are oriented in various directions. Extended bundle sheath (EBS) cells are present in the leaves from the samples of both of the Hawaiian species and the *V. cespitosum* sample from San Bruno Mountain; they are absent in the leaves of the other samples (Table 5, Fig. 4).

DISCUSSION

Phylogenetic Results

Vaccinium sect. *Myrtillus* comprises ca. 12 species mainly distributed across the north-temperate zone

TABLE 4. VARIABLE NUCLEOTIDE POSITIONS IN THE PLASTID REGIONS *MATK* AND *NDHF* AMONG THE SAMPLES OF *VACCINIUM CESPITOSUM*, EVERGREEN “*V. CESPITOSUM*,” AND *V. OVATUM*. The nucleotide with the lowest frequency per site among all samples in the table is indicated in boldface. The GenBank accession numbers of *Vander Kloet 313887* (locality unknown) are AF419703 for *matK*, and AF419735 for *ndhF*; for GenBank and locality information of other samples, see Table 2. Dashes (-) indicate missing data.

Species	Collection	Position								
		<i>matK</i>								
		223	274	423	527	869	870	976	1139	1220
<i>V. cespitosum</i>	<i>Vander Kloet 313887</i>	C	T	C	C	G	G	A	A	G
<i>V. cespitosum</i>	<i>Fritsch 1968</i>	C	T	C	C	G	T	A	A	G
<i>V. cespitosum</i>	<i>McClintock & Menzies s.n.</i>	C	T	C	C	G	T	A	A	G
<i>V. cespitosum</i>	<i>Thomas 10264</i>	C	T	C	C	G	T	A	A	G
Evergreen “ <i>V. cespitosum</i> ”	<i>Fritsch 1967</i>	C	T	C	C	G	T	A	A	G
<i>V. ovatum</i>	<i>Fritsch 1939</i>	T	A	T	A	C	T	C	C	A

in North America and Eurasia, but with *V. cespitosum* extending south into Central America and with four species endemic to Polynesia (Vander Kloet and Dickinson 1999; Powell and Kron 2002; Nelson and Lindstrand 2015). In our plastid analysis, the phylogenetic placements of the samples of *V. cespitosum* newly sequenced for this study, including those from San Bruno Mountain and Point Reyes, agree with their placement in the *V. sect. Myrtillus* clade based on the results of Powell and Kron (2002). In our ITS analysis, they group in a clade that includes all the species of *V. sect. Myrtillus* sampled but which also includes the species from three other sections group as well, without basal resolution, as in Powell and Kron (2002). These other sections all have perennating buds with two partially fused scales, as in the species of *V. sect. Myrtillus*, but are easily distinguished with various morphological characters (Vander Kloet 1988, 2009; Vander Kloet and Dickison 1992). *Vaccinium ovatum* was not included in the study of Powell and Kron (2002). In our analyses, the samples of *V. ovatum*, including the one from San Bruno Mountain, group together in ITS (the plastid analysis included only one sample) and outside of the *V. sect. Myrtillus* clade with moderate to strong support. Sectional sampling is too low in our study to assess its placement relative to other sections. In the ITS trees, the sample of evergreen “*V. cespitosum*” from San Bruno Mountain groups as largely unresolved within the large clade that includes perennating buds with two partially fused scales, but in the plastid trees it groups more specifically with *V. sect. Myrtillus* and particularly with the clade of *V. cespitosum* from the San Francisco Bay Area (the other sample of *V. cespitosum* in the plastid analysis is of unknown provenance).

Support for Hybrid Status of Evergreen “*Vaccinium cespitosum*”

Taken in their entirety, our results provide compelling evidence that evergreen “*V. cespitosum*” from San Bruno Mountain is an intersectional hybrid between the two species of *Vaccinium* on the

mountain, i.e., *V. cespitosum* and *V. ovatum*. In an F1 hybrid, one would typically expect: 1) a plastid haplotype that exactly matches that of at least one putative parent and 2) the presence of 50/50 polymorphisms at sites in the ITS region that differ between the local putative parents (Rieseberg et al. 1993). Our data conform completely to the first expectation in that the evergreen “*V. cespitosum*” plastid haplotype exactly matches the haplotypes of the two samples of *V. cespitosum* from the mountain, and these samples are the only ones in the analysis to do so, including the sample of *V. cespitosum* from Point Reyes and the other sample of *V. cespitosum* included in the study. Regarding the second expectation, however, in the ITS results, no 50/50 polymorphisms were observed; in fact, only one polymorphic site was observed in evergreen “*V. cespitosum*,” a C + T polymorphism, whereas only C was recovered from both putative parents. Nonetheless, although additivity in hybrid ITS region sequences would be unequivocal evidence for hybridization, the lack of such additivity does not necessarily refute a hybrid hypothesis. Rapid homogenization of the ITS region to one of two parental genotypes in hybrids can occur, either through concerted evolution or repeated backcrossing to one parent of an original F1 hybrid (e.g., Buckler et al. 1997; Aguilar et al. 1999; Koch et al. 2003). Moreover, in a study of artificial hybrids the F1 offspring consistently inherited the ITS region ribotype of only one of the parents (Freyre and Tripp 2014). In our data, the sequence of the evergreen “*V. cespitosum*” does not differ unambiguously from the sequences of *V. cespitosum* from San Bruno Mountain. As for the polymorphisms that do differ, the C + T polymorphism in the parents at site 198 versus C in the evergreen “*V. cespitosum*” (Table 3) can be attributed to segregation in one of the parents during meiosis with the hybrid inheriting the C ribotype, whereas the C + T polymorphism that occurs in evergreen “*V. cespitosum*” versus C in the parents may result from the limited population sampling in the parents for our study—the unsampled parent could be (or have been) polymorphic for this site and the polymorphism could have been inherited by the

TABLE 4. EXTENDED.

Position																				
<i>ndhF</i>																				
1607	1622	1719	1787	1914	2007	2036	2154	2156	2186	2234	2237	2249	2298	2306	2324	2357	2468	2540	2582	2609
A	C	G	A	A	G	A	T	G	A	A	G	T	C	A	C	A	T	C	T	C
A	G	C	G	A	G	A	T	T	A	A	G	T	T	A	C	A	T	C	T	C
A	G	G	G	A	A	A	T	T	A	A	G	T	-	-	-	-	T	C	T	C
A	G	G	G	A	A	A	T	T	A	A	G	T	C	A	C	-	T	C	T	C
A	G	G	G	A	A	A	T	T	A	A	G	T	C	A	C	A	T	C	T	C
C	C	G	G	C	G	C	A	T	T	G	A	A	C	T	A	C	A	T	C	T

hybrid. It is also possible that a point mutation could have occurred in the hybrid sample subsequent to the hybridization event.

The hybrid status of evergreen “*V. cespitosum*” is also supported by the sum of gross morphological evidence. Of the 18 characters found to differ among *V. cespitosum*, *V. ovatum*, and evergreen “*V. cespitosum*” (Table 1), 10 are shared by one or the other putative parent; of these, three are shared by *V. cespitosum* (perennating buds toward the shoot apices monomorphic, perennating bud scales 2 and partially fused, and leaf blade glands sparse abaxially) and seven by *V. ovatum* (plants evergreen, glandular trichome position on leaf serrations at ± middle of upper edge of tooth, bracteoles present, calyx lobes well developed, calyx lobe apex ciliate, corolla broadly urceolate, and stamen filaments pubescent). Moreover, six of the 18 characters are more or less intermediate between the putative parents (branchlet trichomes ≤ 0.3 mm long, leaf blades subcoriaceous, leaf blade tertiary veins slightly prominent and obscure on older leaves, inflorescences 1-flowered on branchlets of current season and 2- to 3-flowered fasciculate inflorescences on branchlets of previous season, pedicel articulation with flower present but faint, and anther spurs 0.1–0.8 mm long). The remaining two characters are unique in evergreen “*V. cespitosum*” relative to the putative parents: 1) the branchlets are narrower (0.4–0.6 mm wide, versus 0.5–1.2 mm in *V. cespitosum* and 0.8–2.5 mm in *V. ovatum*), and 2) the pedicels are shorter (0.3–0.9 mm long, versus 2–6 mm in *V. cespitosum* and 2–8 mm in *V. ovatum*). Although this could be considered evidence against a hybrid scenario, it has been demonstrated that novel morphological characters can be produced by hybridization through recombination (Rieseberg et al. 1993), which may well have occurred in this case.

Leaf anatomy also generally supports the hybrid status. Of the characters observed from leaf anatomy, areole size of evergreen “*V. cespitosum*” is intermediate between that of *V. cespitosum* and *V. ovatum* sampled from San Bruno Mountain. The evergreen “*V. cespitosum*” sample also lacks EBS cells, as in *V. cespitosum* from San Bruno Mountain but like *V.*

ovatum from the mountain, and the terminal veinlets of *V. cespitosum* and evergreen “*V. cespitosum*” are generally oriented toward the petiole, whereas those of *V. ovatum* are oriented in various directions. Both of these characters are thus consistent with inheritance of this character by the hybrid from one of the putative parents.

Finally, two sterility characters may also support the hybrid status of evergreen “*V. cespitosum*.” An examination at 64× of the anthers from evergreen “*V. cespitosum*” (*P.W. Fritsch* 2239 BRIT) revealed empty thecae in both open flowers and buds. By comparison, examination at the same magnification of anthers from collections of *V. cespitosum* (e.g., Siskiyou County, California, *P.W. Fritsch* 1925 CAS228590) and *V. ovatum* (e.g., Alameda County, California, *A.A. Beetle* 1654 BRIT) showed thecae with easily visible and fully formed pollen tetrads. The apparent male sterility of evergreen “*V. cespitosum*” conforms to empirical observations for intersectional hybrids obtained by crossing diploid *Vaccinium* species under experimental conditions (Lyrene 2016). This sterility is thought to be caused by the infrequency of normal bivalent pairing between chromosome sets and numerous meiotic abnormalities (Chavez and Lyrene 2010). Although other factors besides hybridization can cause male sterility in plants, the male sterility observed in evergreen “*V. cespitosum*” is consistent with a hybrid origin for this population. Further, the one fruit collected from evergreen “*V. cespitosum*” was devoid of mature seeds, also suggesting inviability due to genetic incompatibility of parental genomes. Although the fruit may merely have been immature when collected, it was dark maroon, suggesting that it was mature or nearly so.

Implications of the Hybrid Status of Evergreen “*V. cespitosum*”

Experimental studies have shown that hybrids can comprise a mosaic of paternal, intermediate, and extreme (novel) characters (Rieseberg 1995). Our data from the species of *Vaccinium* on San Bruno Mountain and their putative hybrid are consistent

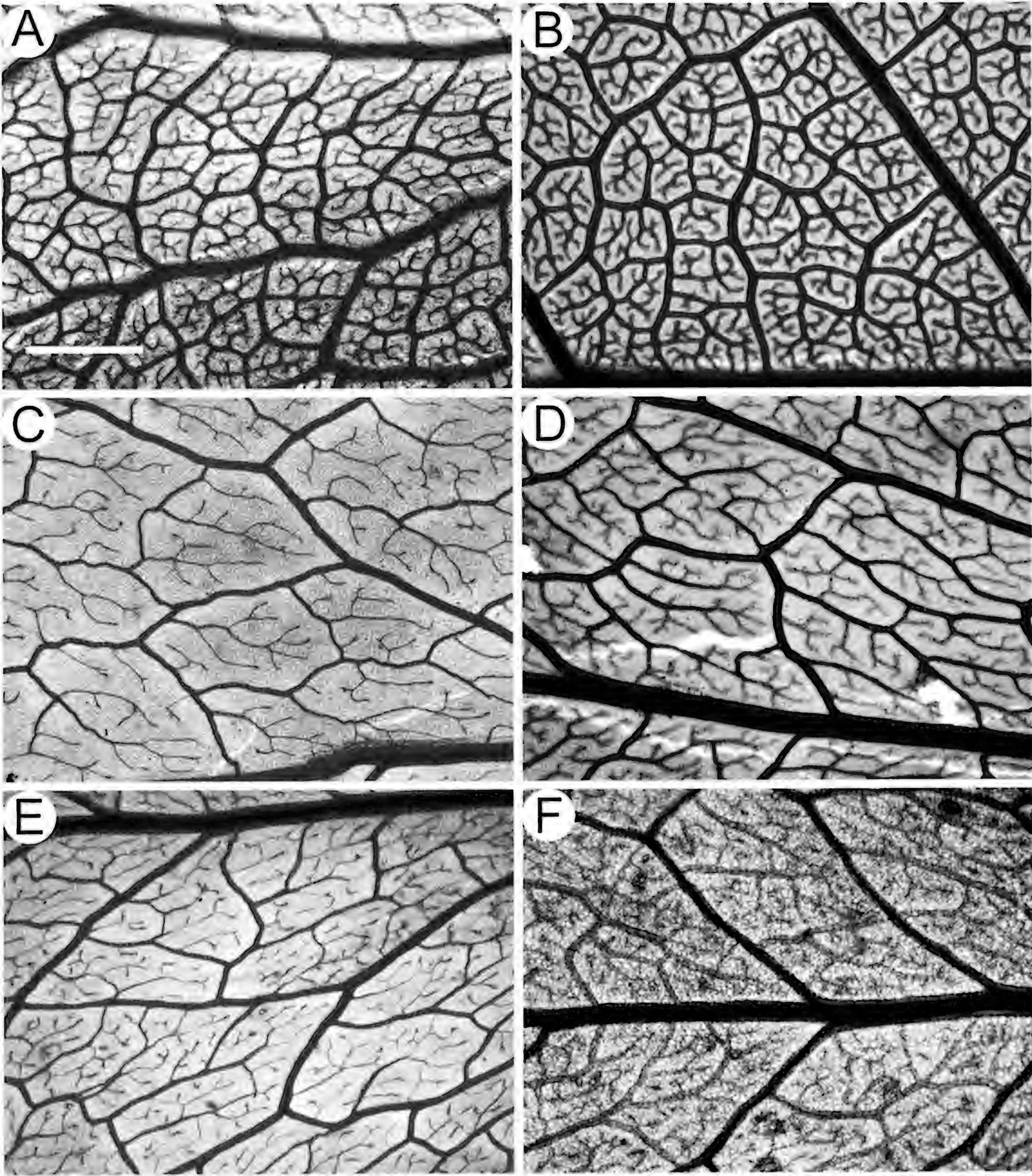


FIG. 3. Leaf blade clearings of selected samples of *Vaccinium* showing veinlet patterns and areole sizes. Scale bar = 1 mm (applies to all subfigures). Leaf apex oriented to the left in all subfigures. A. *V. reticulatum* (Degener 12494), an evergreen Hawaiian species, showing smaller, but prominent, areoles and wide terminal veinlets of various orientations; B. *V. calycinum* (Degener 34837), a deciduous Hawaiian species, showing smaller, but prominent, areoles and wide terminal veinlets of various orientations; C. *V. cespitosum* (deciduous; Fritsch 1968), from Point Reyes, showing larger areoles with narrow veinlets oriented mainly toward the petiole; D. *V. cespitosum* (deciduous; Thomas 10264), from San Bruno Mountain, showing mid-sized areoles and mid-sized veinlets oriented mainly toward the petiole; E. Evergreen “*V. cespitosum*” (Fritsch 2239), from San Bruno Mountain, showing mid-sized areoles with narrow veinlets oriented mainly toward the petiole; F. *V. ovatum* (Allshouse s.n.), from San Bruno Mountain, showing smaller areoles with wide terminal veinlets of various orientations.

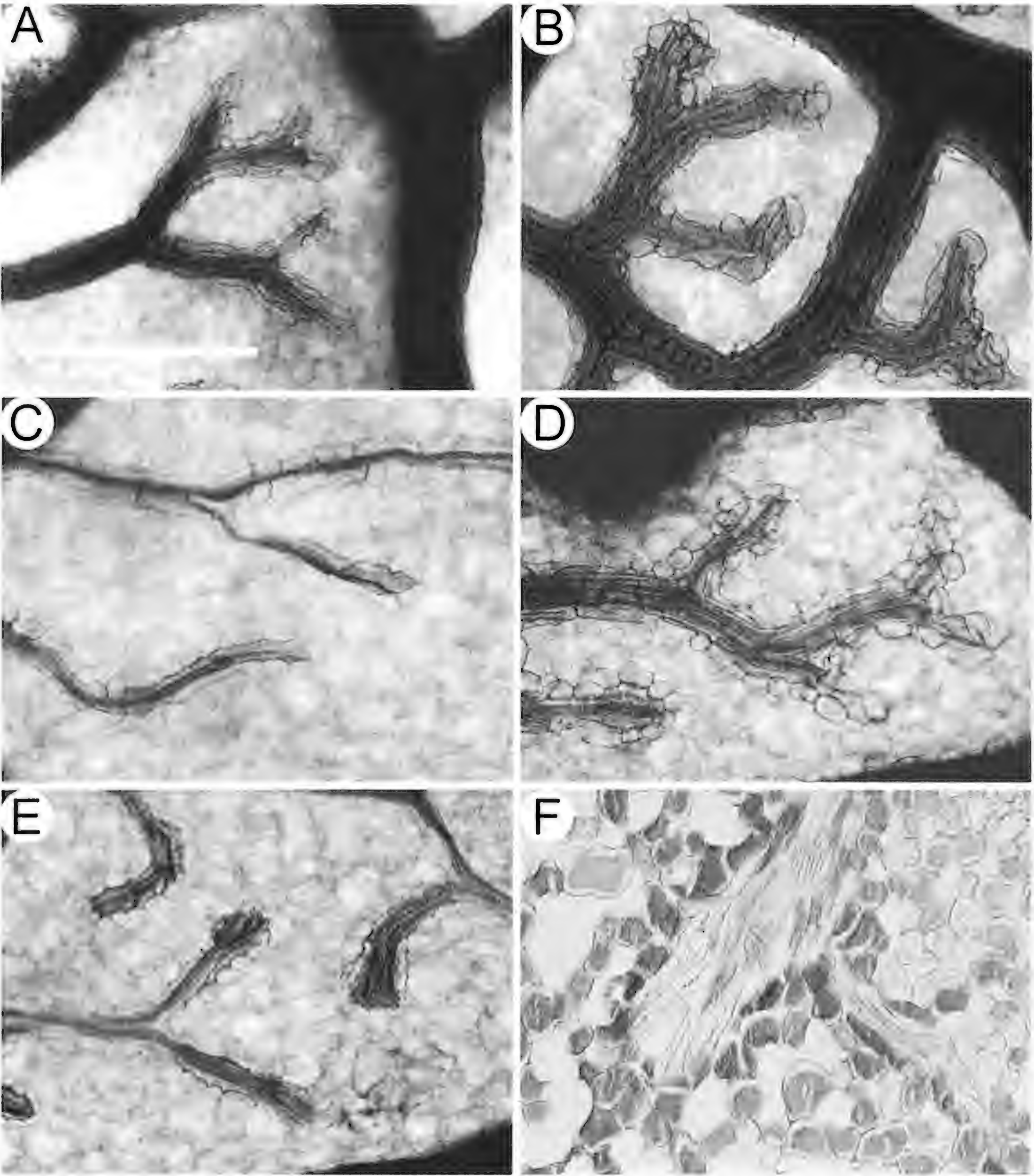


FIG. 4. Leaf blade clearings (A–E) and paradermal section (F) of selected samples of *Vaccinium* showing terminal veinlets. Scale bar = 200 μ m (applies to all subfigures). Leaf apex oriented to the left in all subfigures. A. *V. reticulatum* (Degener 12494), an evergreen Hawaiian species, showing bundle sheath around veinlets and extended bundle sheath (EBS) cells at veinlet endings; B. *V. calycinum* (Degener 34837), a deciduous Hawaiian species, showing bundle sheath around veinlets and EBS cells at veinlet endings; C. *V. cespitosum* (deciduous; Fritsch 1968), from Point Reyes, showing bundle sheath around vein but no EBS cells. Terminal tracheids in veinlets are swollen, illustrating “clubbing;” D. *V. cespitosum* (deciduous; Thomas 10264), from San Bruno Mountain, showing bundle sheath around veinlets and EBS cells at veinlet endings but no “clubbing;” E. Evergreen “*V. cespitosum*” (Fritsch 2239), from San Bruno Mountain, showing bundle sheath around vein but no EBS cells with “clubbing” of the veinlet endings; F. *V. ovatum* (Allshouse s.n.), from San Bruno Mountain, paradermal section showing prominent densely staining sheath parenchyma but no terminal EBS cells.

TABLE 5. COMPARISON OF AVERAGE LEAF VEINLET AREOLE SIZE AND PRESENCE OF EXTENDED BUNDLE SHEATH (EBS) CELLS IN SELECTED REPRESENTATIVES OF *VACCINIUM* SECT. *MYRTILLUS*.

Species	Sample	Location	N	Areole size (mm ² ± S.E.)	EBS cells
<i>V. calycinum</i>	Degener 34837	Hawaii Island, HI	68	0.051 ± 0.004	+
<i>V. cespitosum</i>	Fritsch 1968	Point Reyes, CA	34	1.192 ± 0.117	-
<i>V. cespitosum</i>	Thomas 10264	San Bruno Mt., CA	27	0.295 ± 0.025	+
Evergreen “ <i>V. cespitosum</i> ”	Fritsch 2239	San Bruno Mt., CA	33	0.247 ± 0.024	-
<i>V. ovatum</i>	Allshouse s.n.	San Bruno Mt., CA	14	0.092 ± 0.020	-
<i>V. reticulatum</i>	Degener 12494	Maui Island, HI	69	0.067 ± 0.007	+

with such a mosaic pattern. In the hybrid, 10 gross morphological characters and two leaf anatomical characters are shared with one or the other parent; four other gross morphological characters and one leaf anatomical character show various levels of intermediacy between those of the parents; and two gross morphological characters appear to be novel. The numbers of distinct (i.e., non-intermediate) characters donated by each parent are unbalanced, with two gross morphological and one leaf anatomical character from *V. cespitosum*, versus seven gross morphological and one leaf anatomical character from *V. ovatum*. All the non-intermediate reproductive characters in the hybrid appear to have been derived from *V. ovatum*.

The ability of *Vaccinium* species to hybridize both naturally and in artificial crossing experiments is well known (Camp 1942; Lyrene and Olmstead 2012). Although many intersectional hybrids of *Vaccinium* have been generated in artificial crosses (e.g., Hiirsalmi and Lehmushovi 1982; Lyrene and Olmstead 2012; Ehlenfeldt and Poashock 2014) only two, *V. ×intermedium* Ruthe and *V. cereum* G.Forst., are known to us as occurring under natural conditions. Both of these hybrids, like the hybrid on San Bruno Mountain, include a lineage of *V. sect. Myrtillus* as one of the parents. *Vaccinium ×intermedium*, reported from over 80 localities distributed across much of Europe, is considered to be a diploid-level hybrid between *V. myrtillus* L. of *V. sect. Myrtillus* and *V. vitis-idaea* L. of *V. sect. Vitis-idaea* A.Gray (Ritchie 1955a, b; Ponikierska et al. 2004). Evergreen “*V. cespitosum*” on San Bruno Mountain is likely to have also arisen by a diploid-diploid cross because the two parents are thought to be diploid (Vander Kloet 1988), although the ploidy level of the putative hybrid is unknown. Like evergreen “*V. cespitosum*,” *V. ×intermedium* displays a mixture of distinct and intermediate parental morphological characters, although it does not appear to bear novel characters relative to the parents (Ponikierska et al. 2004).

Vaccinium cereum, from southeastern Polynesia (Marquesas, Society, Tubuai, and Cook Islands; Brown 1935), is thought to be a hybrid between lineages in *V. sect. Myrtillus* from Hawaii and *V. sect. Bracteata* Nakai (Powell and Kron 2002). Hybrid status in this case is based primarily on molecular phylogenetic data, with *V. cereum* grouping with the Hawaiian species in the tree based on plastid data but with a species of *V. sect. Bracteata* in

the tree based on ITS data. Like *V. ×intermedium* and the hybrid on San Bruno Mountain, *V. cereum* exhibits a mixture of distinct parental characters (e.g., perennating buds with two partially fused scales from *V. sect. Myrtillus*, and pedicels articulated with the flowers and pseudo-10-locular ovaries from *V. sect. Bracteata*) and intermediate characters (e.g., bracteoles variably present or absent among populations; Vander Kloet 1996).

The geographic ranges of the parental species of evergreen “*V. cespitosum*” overlap extensively in coastal western North America from central British Columbia to central California (Vander Kloet 1988). Yet, in contrast to *V. ×intermedium*, only one locality of the hybrid between *V. cespitosum* and *V. ovatum* is known. This rarity may be attributable to the unique ecological conditions of the habitat in which the hybrid is found. Although at the macroscale the ranges of the two parental species appear to overlap, at the microscale they are usually separated by habitat at least in California, with *V. cespitosum* found along the margins of various types of wet areas and *V. ovatum* found in dry situations in forests, forest edges, and clearings (Vander Kloet 1988; Wallace 2012; Fritsch personal observation). In this respect, the location of *V. cespitosum* on San Bruno Mountain in dry exposed scrub vegetation would appear at first to be anomalous, except that throughout much of the year, through either rainfall or fog drip, the habitat at Kamchatka Point on San Bruno Mountain is quite wet. This appears to have allowed the two species to grow together at Kamchatka Point and facilitate the potential for hybridization.

Extirpation of *V. cespitosum* from San Bruno Mountain

Vaccinium cespitosum is apparently extirpated from San Bruno Mountain. This species is very rare in the southern end of its range on the coastal side of California. In addition to the San Bruno Mountain locality, the species is known from three other localities in Marin and Sonoma counties. In Marin County it is known from Point Reyes National Seashore south of Abbotts Lagoon (21 May 1991, *W. Follette s.n.* CAS193974!; May 2002, *D. Smith s.n.* CAS193975!; *P.W. Fritsch 1968* CAS471250!), a population that comprises ca. 15 individuals (Fritsch personal observation) and at the Point Reyes Post

Office (July 1903, *A.D.E. Elmer* 4618 CAS193972! and DS-CAS193973!, from which it has apparently been extirpated (Fritsch personal observation). In Sonoma County, it was collected from Perry's Marsh in 1939 and 1940 (*M.S. Baker* 9329 CAS193981! and 9670 CAS193982!, UC877173), but that area has now been completely converted to a spray field for effluent from an adjacent factory, and other uses (Fritsch personal observation), and the species has clearly been extirpated from the site along with most or all of the original marsh vegetation. It has not been collected from Mendocino County.

We suggest three reasons for the extirpation of *V. cespitosum* on San Bruno Mountain, which must have occurred at some point between the last collection of the species in 1979 and our efforts to relocate it from 2012 to 2019. Despite the generally wet conditions at Kamchatka Point, the habitat may still have been too dry to maintain the species, which normally seems to require more permanent ground moisture. Perhaps the species was weakened in years with lower rainfall or less fog than normal, possibly as a result of climate change. Another possibility is the recent lack of fire on the mountain, which otherwise would keep the vegetation low enough for *V. cespitosum* to prevent it being overtopped and crowded out by the mass of dense shrubs; *V. cespitosum* at Point Reyes only achieves a height of ca. 25 cm (from *P.W. Fritsch* 1968 specimen label). Related to this, evergreen "*V. cespitosum*" may have outcompeted *V. cespitosum* at Kamchatka Point. The hybrid forms a patchy but dense shrub layer in the dense scrub vegetation and is taller and more densely branched than typical *V. cespitosum*. Although the ploidy level of the hybrid is unknown, allopolyploids in *Vaccinium* have the ability to sometimes effectively outcompete their ancestral forms (Camp 1942). Further, *V. ×intermedium* spreads faster than its parents because of a higher number of rhizomes than either parent (Ponikierska et al. 2004) and this could have occurred in evergreen "*V. cespitosum*."

The leaves of *V. cespitosum* from San Bruno Mountain are notably more elliptic than in the rest of the California specimens of this species, and their areoles are much smaller than those in the sample from Point Reyes, (averaging 0.295 mm^2 versus 1.192 mm^2), suggesting that the population of *V. cespitosum* from San Bruno Mountain was to some degree geographically and genetically isolated from other populations of this species. Sampling of this population for molecular study is warranted if the species can be rediscovered on the mountain.

Resemblance of the Hybrid to Hawaiian *Vaccinium*

The sum of molecular, morphological, and anatomical data do not support a close relationship between evergreen "*V. cespitosum*" and Hawaiian *Vaccinium*. In the phylogenetic analysis based on plastid data, evergreen "*V. cespitosum*" does not group with the Hawaiian clade. Morphological

characters such as inflorescences with more than one flower, bracteolate pedicels, and pedicels (faintly) articulated with the flowers do not occur in the Hawaiian species. Although these characters do all occur in the hybrid species *V. cereum*, they are thought to have been derived from a lineage in *V. sect. Bracteata* (Powell and Kron 2002). Leaf anatomy also does not support the relationship, with the Hawaiian species differing by their much smaller average areole size, presence (versus absence) of EBS cells at veinlet endings, and terminal veinlets oriented in various directions (versus generally toward the petiole). Thus we conclude that the resemblance of evergreen "*V. cespitosum*" to Hawaiian *Vaccinium* is based on ancestral character similarity and convergence.

Notes on Leaf Anatomy and Morphology

Our measurements of average areole size in the species that were sampled in Powell and Vander Kloet (1997) are similar to those in that study on a relative scale, but are more widely variable. The average size of their sample of *V. cespitosum* was $0.350 \pm 0.119 \text{ mm}^2$ versus our samples from San Bruno Mountain at $0.247 \pm 0.024 \text{ mm}^2$ and from Point Reyes at $1.192 \pm 0.117 \text{ mm}^2$; their samples of *V. calycinum* and *V. reticulatum* were 0.114 ± 0.042 and 0.126 ± 0.051 , respectively (versus ours at $0.051 \pm 0.004 \text{ mm}^2$ and $0.067 \pm 0.007 \text{ mm}^2$, respectively). The reason for the differences in measurements between the two studies is not entirely clear, but one possibility may be because Powell and Vander Kloet measured areole sizes as approximate length and width measurements on areoles that are only rarely rectangular, whereas we used ImageJ to calculate more precise areas of these irregularly shaped objects. The two studies also may have estimated areole closure differently, because sometimes it was difficult to determine whether the veins actually form a closed loop or whether instead a terminal veinlet merely overlaps visually with one of the sides of a veinlet, thus giving the false appearance of being closed. Regardless, the data from the two studies are consistent in that relative average areole sizes are smaller in the Hawaiian species than in the samples of *V. cespitosum*.

TAXONOMIC TREATMENT

Based on the evidence for hybrid status, we recognize the population of evergreen "*V. cespitosum*" as an intersectional hybrid nothospecies, described below.

Nothospecies Description

Vaccinium ×brunoense P.W.Fritsch, sp. nov. (Figs. 5–7).—TYPE: USA, California, San Mateo Co., San Bruno Mountain County Park, W end of San Bruno Mountain, at Kamchatka Point just below

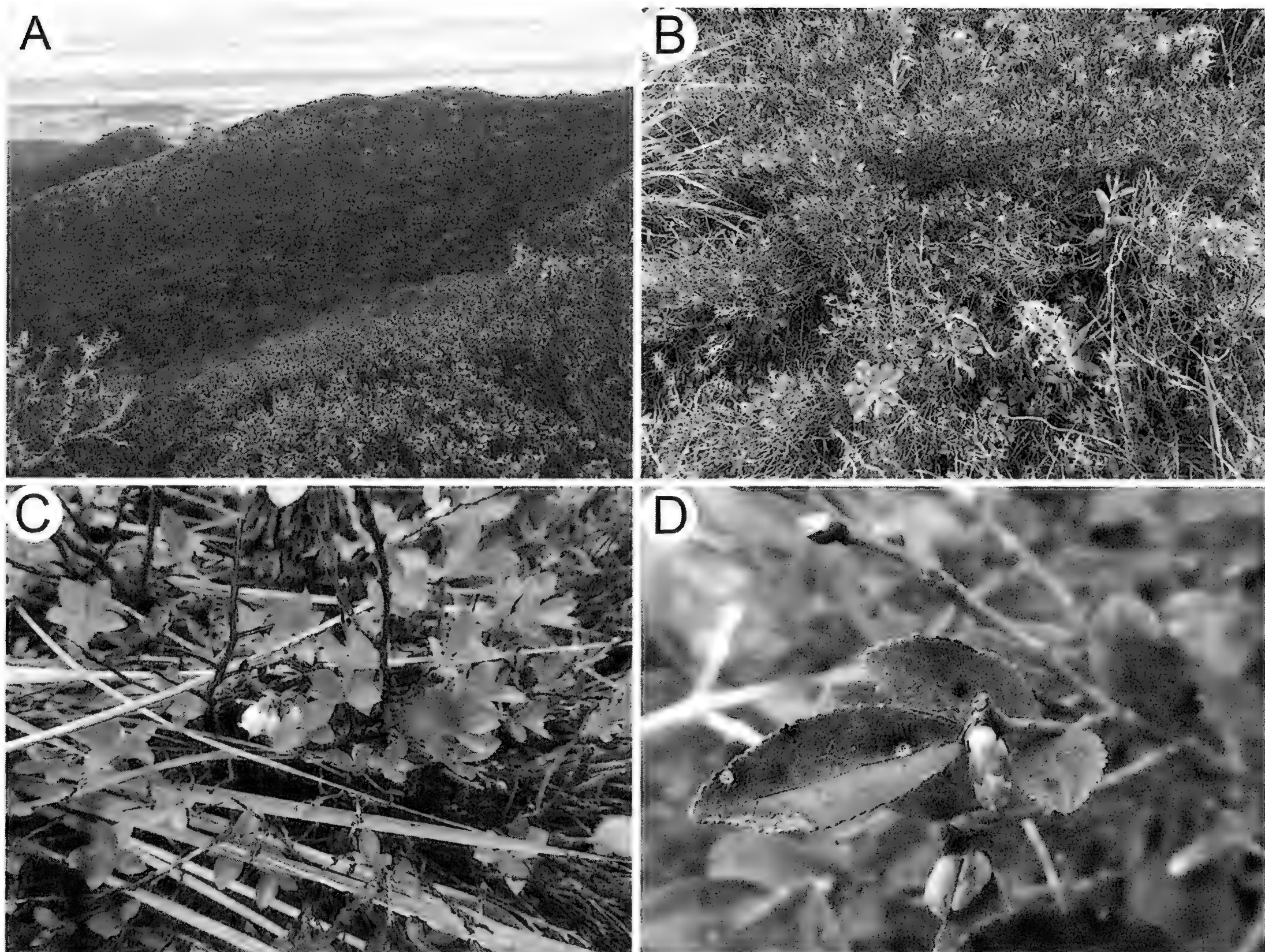


FIG. 5. Images of *Vaccinium ×brunoense* and its habitat. A. Habitat showing northern coastal scrub vegetation covering the western-facing slope of Kamchatka Point on San Bruno Mountain; B. Habit; C. Branchlets with new leaves and flowers; D. Branchlet with flower bud. Photographs: A from David Nelson; B, D from Margo Bors; C from P. W. Fritsch.

Ridge Trail, 37.69014°N, 122.43621°W, 318 m, 26 February 2015 (fl.), P. W. Fritsch 1967 (holotype: CAS1200433 barcode 471249!).

Description. Terrestrial shrublet, evergreen, to 6.6 dm tall, erect, ascending, decumbent, or pendent (on slope), densely branched, clonal through rhizomes. Branchlets grayish brown, reddish brown, or maroon (older branchlets grayish brown), terete and smooth in living condition but drying irregularly ridged, 0.4–0.6 mm wide, evenly puberulent, with trichomes white, ascending-uncinate, variable in length, 0.02–0.3 mm long; axillary perennating buds ovate-deltoid, the larger 1–2.9 mm long, covered by 2 partially fused scales that are light brown or occasionally flushed with reddish pink, scattered hirtellous-puberulent at least apically; pseudoterminal reduced or abortive bud dark brown or black, to 1 mm long, narrowly conical on a narrow stipe (extension of branchlet), stipe and/or bud usually at least slightly divergent from rest of branchlet. Innovations perulate proximally, leafy distally; perules several, ovate to elliptic; leaves 5 to 7. Leaves spirally arranged, petiole green, often flushed reddish

pink, 0.9–1.2 × 0.7–0.8 mm, concave, puberulent or glabrous, leaf blades elliptic, the larger 13–23 × 5.5–10 mm, 1.8–2.1 times as long as wide, subcoriaceous, green adaxially, pale green abaxially, dull to slightly shiny both sides, abaxially with scattered appressed to ascending linear-glandular trichomes at least when young especially along major veins with glandular trichomes 0.12–0.2 mm long, adaxially puberulent at very base especially along midvein otherwise glabrous, base broadly cuneate to subrounded, margin serrulate, 13- to 23-toothed per side, planar or slightly revolute, without sessile glands, often slightly thickened, often flushed maroon, apex acute to subrounded, marginal teeth tipped by translucent to orangish brown linear glandular trichomes 0.1–0.16 mm long oriented toward apex and borne at ±middle of upper edge of each tooth, midvein prominent abaxially, raised adaxially, secondary veins pinnately arranged, planar or raised both sides, 3 to 6 on each side of midvein, anastomosing before reaching margin, tertiary veins abaxially slightly prominent or planar on young leaves, in older leaves obscure, adaxially not visible. Inflorescences either 1-flowered and borne proximally on branchlets of

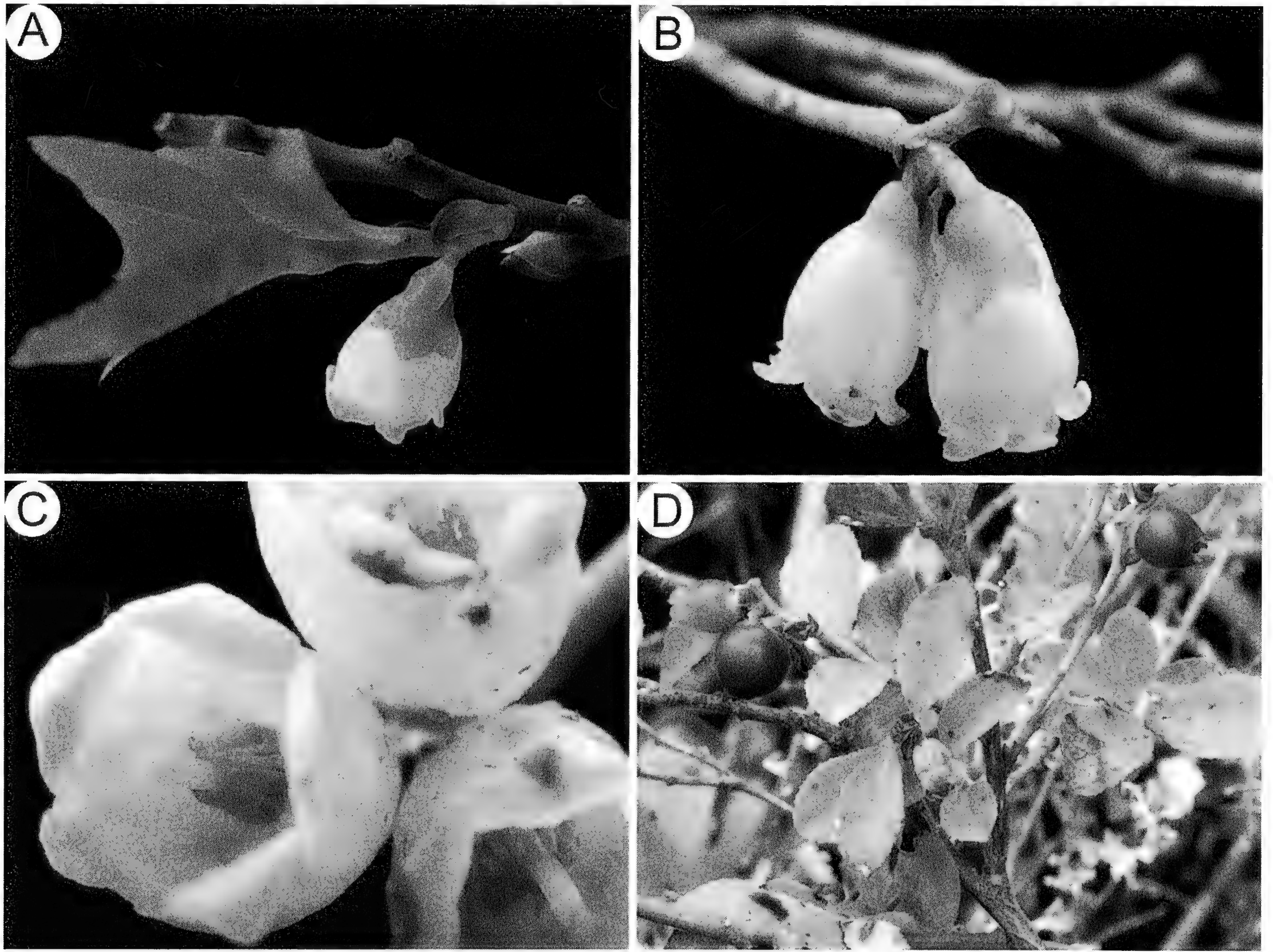


FIG. 6. Images of *Vaccinium* \times *brunoense*. A. Branchlet with a single flower. One of the two bracteoles can be seen covering the calyx tube; B. Branchlet with 3-flowered fasciculate inflorescence; C. Flowers, showing anthers and style; D. Fruiting branchlets. Photographs: A–C from P. W. Fritsch; D from David Nelson.

current season, or either 1- or 2-flowered or 3-flowered in a fascicle on previous year's branchlets; subtending leaf ca. 6×3 mm or (usually) more reduced and bract-like or if bract-like then $2.1\text{--}3 \times 2\text{--}2.2$ mm, caducous, ovate to obovate, cucullate, glabrous, margin glandular-fimbriate, apex acute to rounded. Flowers 5-merous, closed in bud, 5–6 mm long. Pedicels slightly articulated with flowers (especially visible in young fruit), $0.3\text{--}0.9 \times 0.3\text{--}0.5$ mm, glabrous, bracteoles caducous, 2, borne at ca. middle of pedicel, opposite, green often flushed reddish pink, ovate-lanceolate or narrowly elliptic, cucullate, $1.8\text{--}2.5 \times 0.7\text{--}1$ mm, membranaceous, glabrous, margin entire or occasionally glandular, drying strongly inrolled, apex acute to acuminate. Calyx glabrous, tube pale green, cupuliform, $0.7\text{--}1.2 \times 1\text{--}1.2$ mm, smooth in living condition, drying irregularly ribbed, limb erect, 1.3–2.3 mm long, connate portion 0.6–0.9 mm long; lobes 5, pale green often flushed reddish pink, deltoid to ovate-deltoid, $0.7\text{--}1.3 \times 1\text{--}1.3$ mm, margin smooth, apex acute to acuminate, sparsely ciliate. Corolla gamopetalous for most of length, white often slightly flushed pink, broadly urceolate with rounded sides, $4.3\text{--}5 \times 2.4\text{--}3.6$ mm, glabrous,

lobes 5, recurved to slightly reflexed, ovate to deltoid, $0.8\text{--}1.2 \times 1\text{--}1.4$ mm. Stamens 10, inserted at anthesis, 2.5–3.2 mm long, filaments distinct, 1.5–1.7 mm long, margins hirtellous with trichomes to 0.16 mm long, anthers spurred, 1.4–1.9 mm long, eglandular, cells 0.5–0.9 mm long, minutely echinulate, tubules nearly parallel, opening by oblique pores, 0.6–1.2 mm long; spurs borne at base of tubules, \pm straight or upcurved, 0.1–0.8 mm long. Ovary 5-locular; disk flat, glabrous, style included, 3.6–4.2 mm long, glabrous. Fruit broadly ovoid and deep pink when immature, at maturity broadly ovoid to subspherical, dark maroon, ca. 5.5×5 mm, fleshy, shining but with patches of glaucous bloom, with \pm erect persistent calyx.

Additional specimens examined (paratypes). USA. CALIFORNIA. **San Mateo Co.**: San Bruno Mountain County Park, W end of San Bruno Mountain, at Kamchatka Point just below Ridge Trail, 318 m, 30 June 2016 (immature fr.), *D. Allshouse s.n.* (BRIT); *ibid.*, 19 October 2012 (fl.), *P. W. Fritsch 1950* (CAS1130630 barcode 238185); *ibid.*, 28 February 2017 (fl.), *P. W. Fritsch 2239* (BRIT);

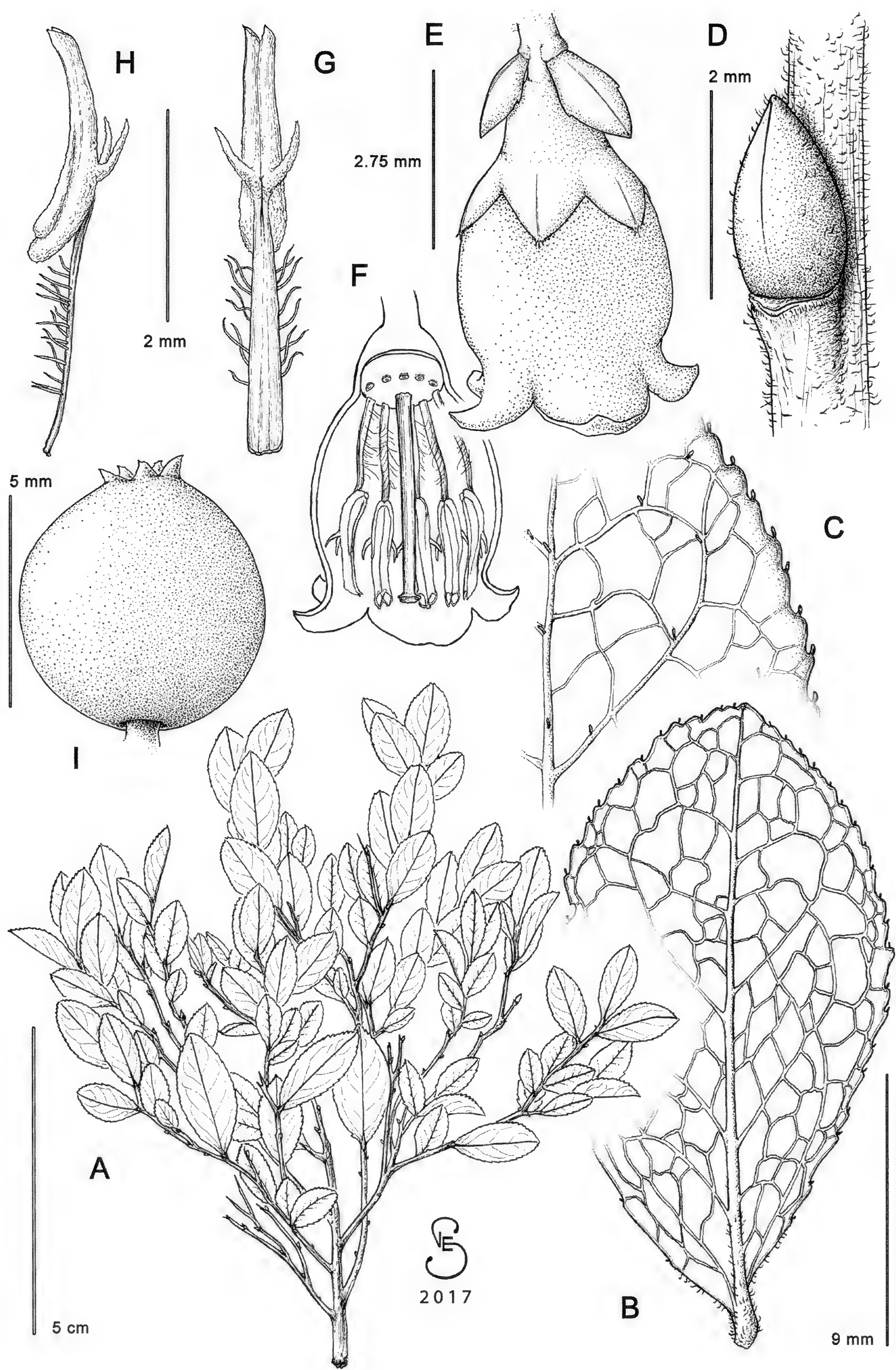


FIG. 7. *Vaccinium* \times *brunoense*. A. Branch; B. Leaf, abaxial side; C. Close-up of leaf blade, abaxial side; D. Segment of stem with perennating bud; E. Pedicel, bracteoles, and flower; F. Flower with part of calyx and corolla cut away to show gynoecium and five of the 10 stamens; G. Stamen, dorsal view; H. Stamen, lateral view; I. Fruit. A, based on *J. H. Thomas 9317A* (CAS; lower individual on sheet); B–E, G, and H, based on *P. W. Fritsch 1950* (CAS); F, based on *P. W. Fritsch 1967* (CAS); I, based on *E. McClintock & P. Reeberg s.n.* (CAS) and images of the living plant. Drawing by Sean Edgerton.

San Bruno Mountain, Kamchatka Point near radio station, 16 February 1963 (st.), *E. McClintock & A. Menzies s.n.* (CAS525478 barcode 199179 *p.p.* [mixed with *V. cespitosum*, this species only in the packet, on which is annotated “June 7, 1979, same locality”]); San Bruno Mountains, W-facing slope of Kamchatka Point, 12 August 1988 (fr.), *E. McClintock & P. Reeberg s.n.* (CAS1024862 barcode 199180 *p.p.* [mixed with *Prunus sp.*]; San Bruno Mountain, near 37°41’N, 122°26’W, 1000–1300 ft., 7 May 1961 (corolla, immature fr.), *J. H. Thomas 9317A* (CAS436403 barcode 199177 *p.p.*, DS458520 barcode 199176 *p.p.* [both mixed with *V. cespitosum*]); *ibid.*, 27 August 1961 (st.), *J. H. Thomas 9709* (DS577992 barcode 199175); San Bruno Mountain, Kamchatka Point near TV tower, ca. 1100 ft., 9 June 1963 (st.), *R. F. Thorne & P. Everett 32214* (DS514654 barcode 199174 *p.p.* [mixed with *V. cespitosum*], RSA174014 and RSA174017 not seen, UC1302442 not seen).

Excluded specimen. The collection *J. H. Thomas 10264* [San Mateo Co., San Bruno Mtn., 1000–3000 ft., near 37°41’N, 122°26’W, 11 September 1963 (st.; DS577566 barcode 199178!)] consists entirely of *V. cespitosum*.

Etymology and suggested common name. The nothospecies is named for San Bruno Mountain, San Mateo County, California, its only known geographic occurrence. We propose the common name San Bruno Mountain Huckleberry.

Distribution, ecology, and phenology. *Vaccinium ×brunoense* is known from only a single population of ca. 200 m² extent just below the top of the ridge on the western slope of Kamchatka Point on San Bruno Mountain, San Mateo County, California. The species grows in northern coastal scrub vegetation, Coyote Brush series (Ford and Hayes 2007) with *Arctostaphylos imbricata* Eastw., *A. uva-ursi* (L.) Spreng., *Baccharis pilularis* DC., *Calamagrostis nutkaensis* (J.Presl) Steud., *Dudleya farinosa* (Lindl.) Britton & Rose, *Eriophyllum staechadifolium* Lag., *Iris douglasiana* Herb., *Maianthemum dilatatum* (Alph.Wood) A.Nelson, *Pteridium aquilinum* (L.) Kuhn, *Toxicodendron diversilobum* (Torr. & A.Gray) Greene, *V. cespitosum* (although now apparently extirpated), and *V. ovatum*. Kamchatka Point is one of the coldest and windiest parts of San

Bruno Mountain (hence the name; McClintock et al. 1968).

Discussion. *Vaccinium ×brunoense* is known to flower in February, August, and October and fruit in August. By comparison, the Jepson Manual treatment of *Vaccinium* (Wallace 2012) reports flowering times of May–July for *V. cespitosum* and March–May for *V. ovatum*. We observed *V. ovatum* flowering on San Bruno Mountain at the same time as *V. ×brunoense* in late February. Two herbarium specimens of *V. cespitosum* were collected in flower on San Bruno Mountain, in May (*J. H. Thomas 9317A*) and June (*E. McClintock & A. Menzies s.n.*). Thus, there could have been a narrow time frame in May when the two parents were flowering at the same time to facilitate crossing. Most flowers of *V. ×brunoense* appear not to develop fruit, so *V. ×brunoense* may have a wider range of fruiting times than indicated here [mature fruit has been seen only in *E. McClintock & P. Reeberg s.n.* and through the personal observation of D. Nelson (photo), both in August].

In the past several years, multiple search efforts to locate additional populations of *V. ×brunoense* have yielded negative results. The number of individuals of *V. ×brunoense* is uncertain due to the clonal habit of the species in which above-ground stems are connected by rhizomes. The plants lie completely within San Bruno Mountain State and County Park and are thus afforded protection. Fires can occur on the mountain, but the extent to which the species is susceptible to fire is unknown. Fruits are rarely observed, perhaps because of seed abortion related to inbreeding depression, as documented for *V. corymbosum* and proposed for other *Vaccinium* species (Krebs and Hancock 1990, 1991). The hybrid may consist of a single F1 clone.

In the most recent key to the California species of *Vaccinium* (Nelson and Lindstrand 2015), *V. ×brunoense* does not key because the first lead of couplet 1 reads “Leaves evergreen, leathery, veins not prominent abaxially; pedicel jointed to flower; filaments ± hairy,” and in *V. ×brunoense* the abaxial surface of the leaf blade has a prominent midvein and the secondary veins can be either planar or raised; only the tertiary veins in older leaves are consistently planar (i.e., not visible). For *V. ×brunoense* to key properly, the initial part of the key can be modified as follows:

1. Plants evergreen; leaf blades subcoriaceous or coriaceous; pedicel at least slightly articulated with flower; stamen filaments pubescent
2. Perennating bud scales 2, partially fused; pedicel 0.3–0.9 mm long *V. ×brunoense*
- 2' Perennating bud scales > 2, distinct; pedicel ≥ 2 mm long
3. Plant < 1.5 dm tall; branchlets slender; leaf generally 7–17 mm long, ± entire; corolla lobes >> tube, reflexed when anthers open *V. macrocarpon*
- 3' Plant 5–30 dm tall; branchlets stout; leaf 20–50 mm long, serrate; corolla lobes < tube, erect to spreading when anthers open *V. ovatum*
- 1' Plants deciduous; leaf blades membranaceous to thick-chartaceous; pedicel not articulated with flower; stamen filaments glabrous

CONCLUSIONS AND FUTURE WORK

We found morphological and molecular support for the hybrid status of an unusual population of *Vaccinium* on San Bruno Mountain: *V. ×brunoense* is likely an intersectional hybrid between *V. cespitosum* of *V. sect. Myrtillos* and *V. ovatum* of *V. sect. Pyxothamnus*. The hybrid exhibits a mixture of distinct and intermediate parental characters, as well as two novel characters. The plastid haplotype and ITS ribotype detected in the hybrid with direct Sanger sequencing were both from *V. cespitosum* on San Bruno Mountain, the latter of which is now extirpated, possibly through being outcompeted by the hybrid.

Further study of *V. ×brunoense* can involve at least four avenues of research. First, it is currently uncertain as to whether the sole known population of *V. ×brunoense* comprises a single individual or multiple individuals because, although rhizomatous growth is confirmed for the species, the extent of such growth is unknown. This could be tested with high-resolution population-genomic data. Second, more phylogenetic data, e.g., with high-throughput DNA sequencing methods, could further assess the hybrid status of the population through the detection of the *V. ovatum* contribution to the hybrid genome. Third, the ploidy levels of the hybrid could be determined and at least *V. ovatum* from San Bruno Mountain (and possibly *V. cespitosum* if herbarium material was adequate) could be confirmed, e.g., with flow cytometry. Finally, further field surveys could be conducted to try to relocate *V. cespitosum* at Kamchatka Point or discover new populations in neighboring areas of San Bruno Mountain or other peaks in the region.

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NEWLY DISCOVERED POPULATIONS OF *ASTRAGALUS TRICARINATUS* (TRIPLE-RIBBED MILKVETCH, FABACEAE) ARE FOUND ON CARBONATE SOILS IN THE SANTA ROSA MOUNTAINS, RIVERSIDE COUNTY, CALIFORNIA

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ABSTRACT

Astragalus tricarinatus A.Gray (triple-ribbed milkvetch) is a rare perennial herb endemic to southern California and is listed as endangered under the Federal Endangered Species Act. Field surveys conducted between 2014 and 2017 relocated *A. tricarinatus* at one of three historical occurrences in the southern portion of its range in Riverside County, California. Surveys in the Chuckwalla Mountains and Orocopia Mountains were not successful in relocating historical occurrences; however, new occurrences were documented in the Santa Rosa Mountains. Plants in the Santa Rosa Mountains were growing on carbonate soils, a substrate that had not been previously associated with *A. tricarinatus*, which typically occurs on metamorphic rock of granitic origin.

Key Words: *Astragalus tricarinatus*, botanical surveys, carbonate, endangered, Fabaceae, floristics, rare plants, Santa Rosa Mountains.

Astragalus tricarinatus A.Gray (triple-ribbed milkvetch) is a short lived perennial herb in the Fabaceae (pea family) endemic to southern California in Riverside and San Bernardino counties. It was listed as endangered under the Federal Endangered Species Act in 1998 due to its limited distribution, small population size, and threats including habitat modification, illegal off-highway vehicle activity, and the presence of exotic species (USFWS 2009; Fraga et al. 2015, CNPS 2019). The core of its distribution is in the foothills of the San Bernardino and Little San Bernardino Mountains, primarily occurring on lands managed by the Bureau of Land Management, The Wildlands Conservancy, and Joshua Tree National Park (Fig. 1; CNDDDB 2019; CCH 2020). Three historical occurrences are disjunct from the core distribution and are located in the Chuckwalla Mountains, Orocopia Mountains, and Agua Alta Canyon in the Santa Rosa Mountains in Riverside County, California. These locations are documented by vague historical references in the herbarium record and literature and have not been observed in over 20 yr.

The occurrence in the Chuckwalla Mountains is based on a collection by Mary F. Spencer on April 6, 1921 (GH366431), and simply states: “Chuckwalla Mountains” as the locality (CCH 2020). The Orocopia Mountains occurrence is based on the Atlas of North American *Astragalus* by Rupert Barneby (1964), wherein he describes the distribution of the species as “known only from around the north end of Coachella Valley, from Whitewater and Morongo Wash across the south face of the Little San Bernardino Mountains to the Orocopia Range.” The Santa Rosa Mountains occurrence is based on a specimen collected by J.M. Stewart in 1985 from a

single individual in Agua Alta Canyon (UCR38117; CCH 2020).

Here we provide a summary of findings from recent botanical surveys for *A. tricarinatus* at three historically documented occurrences at the southern edge of its range in Riverside County, California. The surveys took place in an effort to provide up-to-date information regarding the distribution, abundance, habitat preference, status, and threats to *A. tricarinatus* in the portion of its geographic range where it is less well known, and to inform management and long-term conservation of the species.

METHODS

Focused surveys for *A. tricarinatus* were conducted between 2014 and 2017 at three historical locations: the Chuckwalla Mountains, the Orocopia Mountains, and the Santa Rosa Mountains. Prior to field surveys we examined aerial imagery available online via Google Maps (Google, Mountain View, CA) to identify potential habitat in all three areas. The soil that *A. tricarinatus* most often occurs on has a distinctive grey-green color and can often be identified from satellite imagery (Fraga et al. 2012). However, our remote-sensing exercise did not locate obvious potential locations to survey in the three focal areas. Therefore, we identified field survey locations based on areas where botanists would have collected plants in the past, and areas identified as potential habitat by the lead author from on-the-ground surveys. To increase chances of sight recognition in the field and proper identification, most surveys were conducted between the months of March and May when *A. tricarinatus* is most likely to be in flower and fruit. When *A. tricarinatus* was

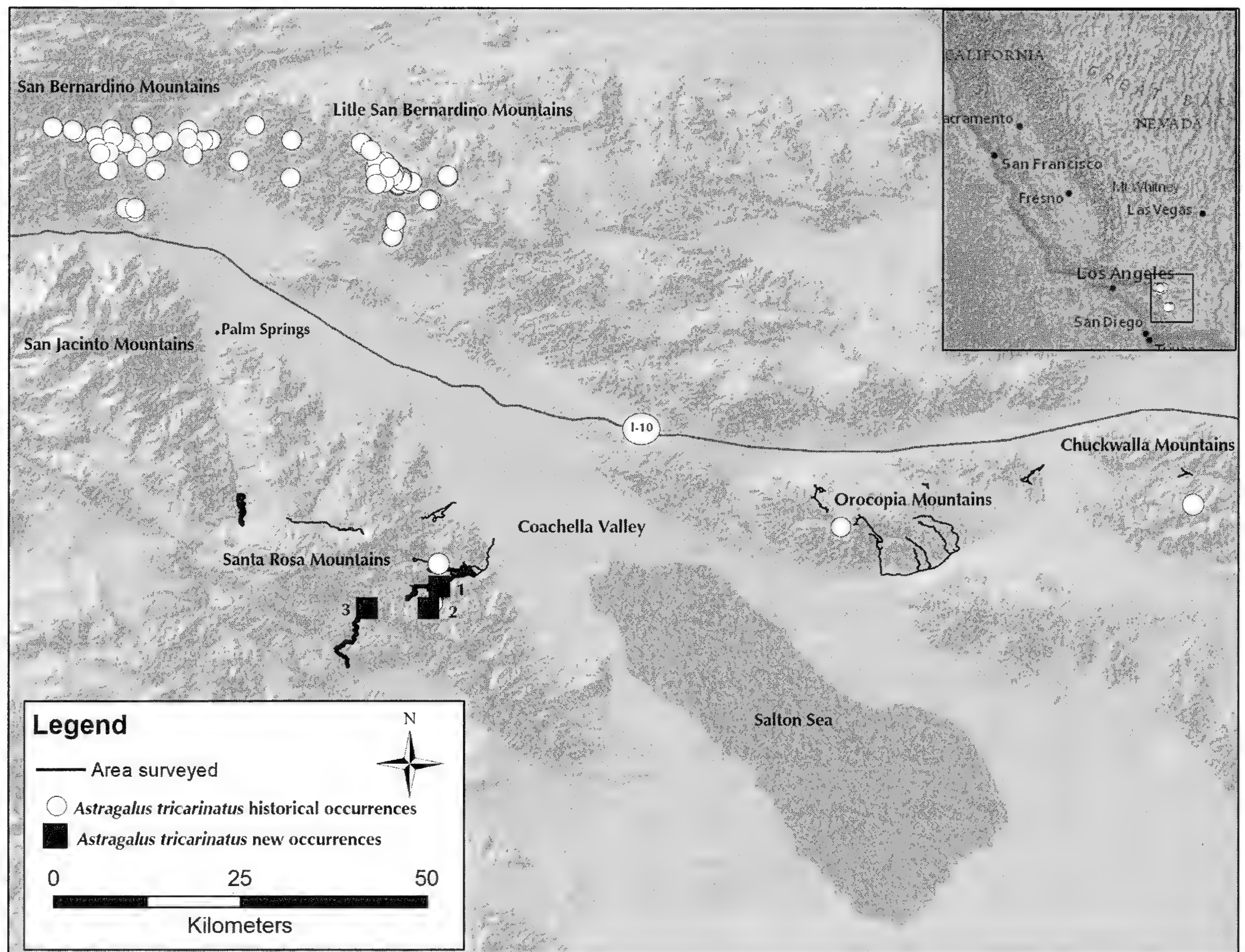


FIG. 1. Distribution map of *Astragalus tricarinatus* (triple-ribbed milkvetch). The known range of *A. tricarinatus*, including historical occurrences, is denoted by white circles. The areas surveyed are denoted by black lines. New occurrences discovered as a part of this study are denoted by dark gray squares and are numbered by ID according to Table 1.

found, rocks were sampled to aid in substrate identification. We conducted an acid test on a rock that was collected from a site where *A. tricarinatus* occurs, in the Santa Rosa Mountains, by placing a drop of dilute (10%) hydrochloric acid to determine if carbonate was present.

The majority of the areas we identified for surveys are located on land managed by the Bureau of Land Management (BLM) and is designated wilderness according to the Wilderness Act of 1964. Wilderness designation and a lack of open roads made surveys difficult because areas that historically had road access can now only be accessed by covering several miles on foot. In addition, historical location information for *A. tricarinatus* in the Orocopia and Chuckwalla Mountains is extremely vague; therefore, we needed to conduct field surveys across a wide area to identify potential habitat. During all surveys we recorded detailed habitat and locality information, including associated species, substrate, slope, aspect, latitude, longitude, and elevation.

RESULTS

Chuckwalla Mountains Wilderness Surveys

The Chuckwalla Mountains Wilderness is 12,800 acres (200 sq. mi.) in area, and is composed primarily of undivided Precambrian gneiss (southwest), a Precambrian igneous and metamorphic rock complex, and Mesozoic granite (northeast; Jennings 1967; Jennings et al. 2010). This mountain range is very rugged, and botanical documentation is sparse with less than 1000 herbarium vouchers collected from the region prior to this study (CCH 2020). Most botanical documentation from the Chuckwalla Mountains is from the vicinity of Corn Spring, Aztec Well, the north side of the Chuckwalla Mountains south of Interstate 10, and the west side of Red Cloud Mine. Mary Spencer, the botanist who documented *A. tricarinatus* in the Chuckwalla Mountains, collected it at a time that overlaps with collections of Edmund Jaeger, thus we infer that Spencer and Jaeger were collecting together (CCH 2020; Jaeger 1982). Jaeger specified the location of

Aztec Well on his labels; therefore, the majority of our surveys were conducted in that area. Other historically visited sites, such as Corn Springs and the northern section of the range, were also surveyed. *Astragalus tricarinatus* was not relocated in the Chuckwalla Mountains; however, we identified several sites in the vicinity of Aztec Well with open exposed slopes that appear to contain suitable habitat for this species. Therefore, we recommend that future surveys for *A. tricarinatus* be conducted in these areas identified by our surveys.

Orocopia Mountains Wilderness Surveys

At 50,960 acres (80 sq. mi.) the Orocopia Mountain Wilderness is large and geologically diverse. Rock types in the region including gneiss, granite, schist and a variety of sedimentary rocks, such as conglomerate, limestone, and dolomite (Dibblee and Minch 2008a; Jennings et al. 2010). The Orocopia Mountains have seen little botanical documentation, with less than 600 herbarium vouchers that have been collected in the region. Most of these were collected at the margin of the mountains around Salt Creek Wash and along Interstate 10 (CCH 2020). Prior to wilderness designation, there were several roads running south from Interstate 10 to Salt Creek Wash that have since been closed. These routes are now limited to foot traffic for accessing the interior portions of the range. We conducted targeted surveys at several springs and mines along these old routes in the Orocopia Mountains because they historically served as botanical collecting sites. We did not locate *A. tricarinatus* in the Orocopia Mountains; however, we identified several locations in the central portion of the range that could serve as potential habitat.

Santa Rosa Mountains Wilderness Surveys

The vicinity of Agua Alta Canyon in Santa Rosa Mountains is primarily composed of Cretaceous quartz diorite mixed with Precambrian metamorphic rocks, with pockets of Quaternary alluvium, and Mesozoic metasedimentary rocks including mica schist to phyllite and gneiss (Dibblee and Minch 2008b; Jennings et al. 2010). The Santa Rosa Mountains have seen very little botanical documentation due to limited access. Less than 1000 herbarium specimens had been collected in the region prior to this study (CCH 2020). We encountered several access issues in the Santa Rosa Mountains, including roads that are blocked by agricultural fields, private property at the mouth of Martinez Canyon, and difficult terrain. Many canyons in the region feature dry falls that require climbing gear to traverse. The vicinity of Martinez Canyon and Agua Alta Canyon were the primary areas for targeted field surveys based on the historical specimen of *A. tricarinatus* collected in 1985 (Fig. 1).

We did not re-locate *A. tricarinatus* at the historical site in Agua Alta Canyon. We did, however, locate it at three previously undocumented occurrences. The first occurrence is in a large side canyon off of Martinez Canyon, ca. 4 km south of Agua Alta Canyon (Fig. 1; Table 1). Five mature individuals were found growing in the canyon bottom scattered over approximately 1.6 km. Upslope we identified a second occurrence consisting of several mature individuals, and a number of seedlings (Table 1). The second population occurs on a very steep slope composed of carbonate rock (Fig. 2). This is the first time, to our knowledge, that *A. tricarinatus* has been found growing on carbonate soils. We confirmed the presence of carbonate by conducting an acid test on a rock sampled from the site.

We returned to the Martinez Canyon occurrence one year later in March of 2017 and found that four of the five mature individuals previously documented in the canyon had died. We also returned to the second population located upslope from Martinez Canyon, and observed that the seedlings we observed in 2016 did not survive to maturity. Five newly germinated seedlings were found. The mature individuals we observed in 2016 were alive and reproductive (in flower and fruit), and we documented additional mature individuals not found in 2016, in addition to two dead adults that we did not identify in 2016. Based on these surveys, the total estimated population size at the occurrence upslope from Martinez Canyon is 10 individuals.

The observation of *A. tricarinatus* on carbonate soils in 2016 provided an expanded search image for potential habitat in the Santa Rosa Mountains. With added information, several new locations were targeted for surveys in the spring of 2017. As a result, one additional occurrence was documented on the ridge west of Rabbit Peak and north of Rockhouse Canyon and Buck Ridge (Fig. 1). This occurrence is significant for two reasons. First, like the Martinez Canyon occurrence, it was on carbonate soils. Secondly, it documents *A. tricarinatus* in a new watershed. This watershed occurs on the south side of the Santa Rosa Mountains in the Clark Lake watershed, which contributes to the greater Salton Sea Basin. This remote occurrence currently represents the southernmost extent of the range for the species. It was difficult to access due to the steep and rugged terrain; therefore, we had limited time to survey the area. Two reproductive individuals and one vegetative individual were observed in a rocky canyon bottom below steep carbonate slopes. There is potential habitat in this region that should be surveyed more thoroughly. The use of a drone to survey the rugged terrain would also be advantageous at this site.

Several other locations were searched, unsuccessfully, in the Martinez Canyon watershed. Slopes underlain with carbonate substrate were observed (33.48783°, -116.27119°), but slopes within the

TABLE 1. OCCURRENCES DOCUMENTED AS A PART OF THIS STUDY. All locations are in the Santa Rosa Mountains, Santa Rosa Wilderness, Riverside County, California and were documented in 2016 and 2017.

ID	Collection #	Location	Elev (m)	Habitat	Population information
1	Bell 9129	Side canyon off of Martinez Canyon, approximately 6.5 air miles southeast of the summit of Martinez Mountain. 33.49247° , −116.26333°	581	Very steep carbonate slopes and cliff faces with <i>Argyrochosma jonesii</i> (Maxon) Windham, <i>Arctostaphylos glauca</i> Lindl., <i>Astragalus coccineus</i> Brandegee, <i>Cercocarpus betuloides</i> Nutt., <i>Crossosoma bigelovii</i> S.Watson, <i>Galium stellatum</i> Kellogg, <i>Eriogonum fasciculatum</i> Benth., <i>E. wrightii</i> Benth., <i>E. saxatile</i> S.Watson, <i>Juniperus californica</i> Carrière, <i>Peucephyllum schottii</i> A.Gray, <i>Pleurocoronis pluriseta</i> (A.Gray) R.M.King & H.Rob, <i>Salvia eremostachya</i> Jeps., <i>S. apiana</i> Jeps., and <i>Senegalia greggii</i> (A.Gray) Britton & Rose.	A single individual growing in rocky canyon bottom
2	Bell 9214	Side canyon off of Martinez Canyon, approximately 7 air miles southeast of the summit of Martinez Mountain. 33.47716° , −116.27109°	861	Very steep carbonate slopes and cliff faces with <i>Argyrochosma jonesii</i> , <i>Arctostaphylos glauca</i> , <i>Astragalus coccineus</i> , <i>Cercocarpus betuloides</i> , <i>Crossosoma bigelovii</i> , <i>Galium stellatum</i> , <i>Eriogonum fasciculatum</i> , <i>E. wrightii</i> , <i>E. saxatile</i> , <i>Juniperus californica</i> , <i>Peucephyllum schottii</i> , <i>Pleurocoronis pluriseta</i> , <i>Salvia eremostachya</i> , <i>S. apiana</i> , and <i>Senegalia greggii</i> .	Ca. 10 individuals, and a number of seedlings.
3	Bell 10,325	North of Rockhouse Canyon, approximately 7 air miles northwest of Rabbit Peak. 33.47170° , −116.35082°	1211	Narrow rocky canyon with <i>Agave deserti</i> Engelm., <i>Brickellia desertorum</i> Coville, <i>Bromus rubens</i> L., <i>Cercocarpus betuloides</i> , <i>Ephedra</i> sp., <i>Eriogonum wrightii</i> , <i>Erodium cicutarium</i> (L.) Aiton, <i>Galium stellatum</i> , <i>Juniperus californica</i> , <i>Leptosiphon floribundus</i> subsp. <i>hallii</i> (Jeps.) J.M.Porter & L.A.Johnson, <i>Nolina parryi</i> S.Watson, <i>Phacelia cryptantha</i> Greene, <i>Pinus monophylla</i> Torr. & Frém., <i>Prunus fremontii</i> S.Watson, <i>Quercus cornelius-mulleri</i> Nixon & K.P.Steele, <i>Salvia eremostachya</i> , and <i>S. apiana</i> .	Three individuals observed

survey area were not as steep or exposed as the other known locations. A second location in the region surrounding Martinez Canyon supported suitable habitat (33.48222°, −116.30145°) including very steep, open, and exposed carbonate slopes, but did not reveal any *A. tricarinatus*. The terrain was quite difficult to access, thus survey time was limited in this area. This location should be revisited, but may require additional resources such as the use of a drone, and climbing gear.

DISCUSSION AND RECOMMENDATIONS

Chuckwalla Mountains Wilderness

Spencer and Jaeger were friends and traveling companions (Jaeger 1982) and it appears that Spencer may have been collecting plants with Jaeger

when she collected *A. tricarinatus* (CCH 2020). They do not mention other collectors on their herbarium specimen labels, but this was not uncommon at that time. Jaeger’s collections from the Chuckwalla Mountains indicate that he collected plants in vicinity of Aztec Wash and Well, which is more specific than Spencer’s voucher information which simply states “Chuckwalla Mountains.” We assume that if Spencer and Jaeger collected together, her collection of *A. tricarinatus* would have been from the area near Aztec Well or Wash. We recommend that future surveys of *A. tricarinatus* in the Chuckwalla Mountains focus on the region of Aztec Well; in particular, the Precambrian rocks north of Aztec Well and around upper Irish Wash. Our surveys thus far have reached the edges of the Precambrian gneiss, but most areas we surveyed were in Mesozoic granite.

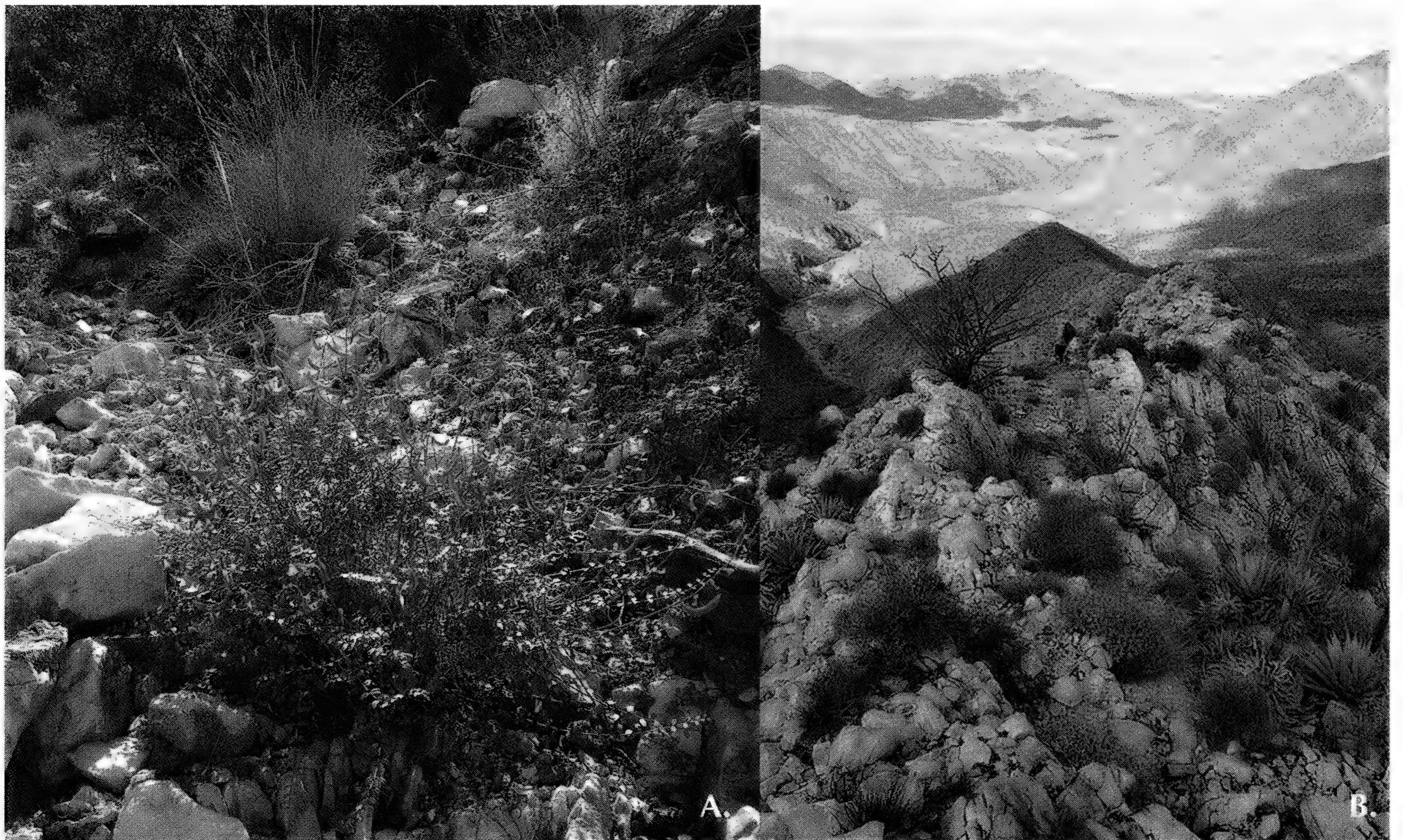


FIG. 2. *Astragalus tricarinatus* in fruit and its habitat. A. *Astragalus tricarinatus* in fruit in the Santa Rosa Mountains. B. Habitat of *Astragalus tricarinatus* in the Santa Rosa Mountains. In the Santa Rosa Mountains, it occurs on carbonate substrate and steep and rugged terrain.

Orocopia Mountains

Barneby noted that the global distribution of *A. tricarinatus* was known from Whitewater to the Orocopia Mountains, but unfortunately he did not cite a voucher specimen referencing a location in the Orocopia Mountains (Barneby 1964). Barneby was known for being a meticulous taxonomist and thorough in his work; therefore, any mention of a geographic location was generally verified by a voucher specimen (Gary Wallace, California Botanic Garden, personal communication). It is possible that there exists or existed a specimen of *A. tricarinatus* from the Orocopia Mountains that is not databased, or was lost. Barneby also states “The whole range of *Astragalus tricarinatus* extending from the Morongo Wash region to the hills northeast of Mecca, has a diameter of about fifty miles.” The hills northeast of Mecca would presumably be the Mecca Hills which make up the western boundary of the Orocopia Mountains. Based on prior surveys in the region, we do not believe there is much potential habitat for *A. tricarinatus* in the Mecca Hills. However, there are a few locations with open exposed slopes that could provide habitat. For instance, the Precambrian gneiss in the Mecca Hills near Painted Canyon (Dibblee and Minch 2008a).

The Orocopia Mountains are geologically diverse and further explorations should be conducted in years with ample rain to increase the chance of observing living individuals. A potential area to

survey is the large unnamed canyon east of Orocopia Canyon and 2.5 miles east of Orocopia Vertical Angle Benchmark (VABM) 3815. This canyon historically had access via a road and contains several rock types (Dibblee and Minch 2008a). The area east of a large unnamed canyon in the center of the range to Red Canyon, is also worthy of exploration due to the diverse geology that occurs there. The north-facing slopes at the upper elevations of the northwestern portion of the range could also provide habitat for *A. tricarinatus*.

Santa Rosa Mountains

As a result of this study, we now have current locality, habitat, and threat information for *A. tricarinatus* in the Santa Rosa Mountains. Further surveys in the region should target areas near known occurrences with similar habitat, including steep, exposed, carbonate rock outcrops, as well as the upper reaches of the Santa Rosa Mountains above Agua Alta Canyon. The Agua Alta Canyon occurrence may be a waif washed down from a large population at higher elevation. Other potential areas to survey include the vicinity of Martinez Mountain, the upper reaches of canyons on the south side of Martinez Canyon, and north of Clark Valley/Rockhouse Canyon where carbonate soils may be present. We documented *A. tricarinatus* growing in open areas on very steep slopes composed of carbonate rock with little accumulation of organic material

(Fig. 2). The plants appear to be growing in cold air drainages near the lower end of the pinyon belt.

Regarding the historical occurrence at Agua Alta Canyon, it should be noted that while we did not relocate plants at this location, it does not mean that *A. tricarinatus* no longer occurs there. Additional surveys should be conducted upslope from Agua Alta Canyon, targeting potential habitat on carbonate slopes and outcrops.

Threats

As a result of this project, three previously undocumented occurrences have been documented and have expanded our knowledge on the distribution, and habitat preferences of the species. Despite this new information, the additional occurrences do not considerably change the status of the species in terms of its global population size, vulnerability, and assessment of threats. The three newly documented occurrences in the Santa Rosa Mountains add anywhere between ten to twenty individuals to the global population count, which will vary from year to year depending on precipitation and other factors. Climate change, small population size, habitat modification, and invasive plant species have been listed as threats to *A. tricarinatus* (Fraga et al. 2012, Heintz et al. 2018). Over 70% of all known documented occurrences are small (i.e., <15 individuals), leaving these populations vulnerable to stochastic events (Fraga et al. 2015). In addition, we observed high mortality (up to 80%) of plants occurring in the Santa Rosa Mountains. Climate change may be a significant threat to this highly restricted species, especially due to its limited range, small population size, narrow habitat requirements, and life history (e.g., plants are short lived). Many plant species are predicted to respond to climate change by shifting their ranges (Anacker et al. 2013; Mason et al. 2015; Still et al. 2015), but individuals of *A. tricarinatus* in the Santa Rosa Mountains appear to be edaphically restricted, similar to occurrences across the range of the species. Species such as *A. tricarinatus* may be unable to migrate or adapt to rapidly changing conditions. Changes in precipitation quantity and timing could have a significant impact on germination and plant survival; therefore, given our observations of small population size, and high mortality, climate change may present a significant risk of local extinction.

Additional Recommendations for Future Surveys

Astragalus tricarinatus habitat in the vicinity of Whitewater Canyon is found mostly in areas mapped as Precambrian quartz diorite and quartz monzonite gneiss. There is also Tertiary Coachella fanglomerate and Pleistocene alluvium in the area (Dibblee 1967). The new localities in the Santa Rosa Mountains were found in an area mapped as mixed Precambrian gneiss and Mesozoic granite, and plants were

documented on carbonate rock (Jennings et al. 2010). Given that *A. tricarinatus* appears to be fairly substrate specific (Fraga et al. 2015), or at least occurs on well drained soils with low competition, and most occurrences have been found in areas mapped as Precambrian gneiss, it may be useful to target areas mapped as gneiss in future surveys.

The documentation of the Chuckwalla and Orocopia Mountains locations, in particular, are fairly old and nonspecific. While it is worthwhile to survey areas previously targeted by botanists, it is also possible that *A. tricarinatus* no longer occurs in the same location where it was last collected, especially due to the transient nature of some populations (Fraga et al. 2015). However, *A. tricarinatus* has strong potential to occur in several locations, therefore, it is not only important to re-survey previously documented historical locations, but also adjacent regions with potential habitat. Based on our assessment of the regions where *A. tricarinatus* was historically collected in the Chuckwalla Mountains, Orocopia Mountains, and the Santa Rosa Mountains, it appears that each mountain range does have potential habitat for this species that should be considered for future survey efforts.

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HYDRAULICS OF *PINUS* (SUBSECTION PONDEROSAE) POPULATIONS ACROSS AN ELEVATION GRADIENT IN THE SANTA CATALINA MOUNTAINS OF SOUTHERN ARIZONA

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ABSTRACT

The ability of plants to tolerate and recover from periodic water stress affects their competitive ability, survival, and distribution, leading to shifts in plant communities as environmental conditions change. We investigated the hydraulic traits of two closely related *Pinus* taxa to assess population and taxonomic variability in plant hydraulic traits. We hypothesized that traits would vary with elevation but exhibit similar traits where taxa co-occurred. We measured predawn and midday leaf pressure potential (Ψ_p) across three seasons, xylem specific hydraulic conductivity (K_s), and vulnerability to xylem embolism (P_{50}). These were measured on *Pinus ponderosa* var. *brachyptera* (Engelm.) Lemmon that occurred at a high elevation site (2770 m), *P. arizonica* Engelm. at a low elevation site (2135 m), and both species where they co-occurred at the mid-elevation site (2475 m) in the Santa Catalina Mountains of southern Arizona. Plants from the high elevation site had the least negative Ψ_p and the highest K_s . The two taxa differed from one another when compared between the high and low elevation sites, but they were not different where they co-occurred. The two *Pinus* taxa show plasticity in their hydraulic traits across sites. Conditions across the elevational gradient appear to lead to a convergent solution in hydraulic traits for these taxa where their ranges overlap but differences in traits where they do not overlap. Increasing aridity in the region could lead to shifts in suitable habitat, reduced water transport ability at range margins, and shifts in population distributions.

Key Words: cavitation resistance, elevation, embolism, hydraulic traits, Ponderosae, xylem conductivity, water potential.

Long-lived woody plants persist for long periods of time and through varying conditions. The abilities of plants to efficiently transport water and to withstand hydraulic transport failure in response to periodic water stress are important determinants of their ability to tolerate the long-term extreme conditions of a particular site and to successfully compete for limited resources. Resistance to water stress-induced xylem embolism is an important plant functional trait that varies across broad scales and is linked to functional differences among ecosystems (Maherali et al. 2004; Choat et al. 2012) and plant communities (Jacobsen et al. 2007; Hacke et al. 2009). Across the ranges of individual species, variation in embolism resistance has been found between populations in some species (Mencuccini and Comstock 1997; Kavanagh et al. 1999; Kolb and Sperry 1999; Pratt et al. 2012; Jacobsen et al. 2014). Efficiency of hydraulic transport is also an important trait that varies across landscapes and ecosystems (Gleason et al. 2016) and may vary between populations growing in different environmental conditions (Maherali and DeLucia 2000).

However, relatively little is known about hydraulic functional plasticity and population variation, and this area of study was recognized as particularly

limited in two recent reviews (Jacobsen et al. 2014; Anderegg 2015). These have also been identified as key areas of inquiry where more information is required to better understand plant response to drought (Choat et al. 2012; Anderegg 2015). Species plasticity and population variability may also dramatically impact predictions of species responses to climate change (Valladares et al. 2014), which could alter adaptation and mitigation strategies.

Within *Pinus*, evidence of population divergence in hydraulic traits has been mixed. Some studies have found that populations of Scots pine (*P. sylvestris* Baumg.) (Martínez-Vilalta and Piñol 2002; Martínez-Vilalta et al. 2009) and maritime pine (*P. pinaster* Loudon) (Lamy et al. 2014) have little variation in hydraulic conductivity or cavitation resistance. In contrast, studies on other *Pinus* species have found that some hydraulic traits do appear to vary among populations, including in Aleppo pine (*P. halepensis* M.Bieb.) (Tognetti et al. 1997; David-Schwartz et al. 2016), ponderosa pine (*P. ponderosa* Douglas ex. P. Lawson & C. Lawson) (Maherali and DeLucia 2000), and Canary Island pine (*P. canariensis* C.Sm. ex DC.) (López et al. 2016). Furthermore, several studies have confirmed a link between drought and pine species distributions, suggesting that hydraulic

traits may be important determinants of species and populations distributions and structure (Allen and Breshears 1998; Bigler et al. 2006; McDowell et al. 2009).

Within the Santa Catalina Mountains of southern Arizona, two closely related *Pinus* taxa within the subsection Ponderosae turn over along a topographical, microhabitat, and elevational gradient (Kearney and Peebles 1961; Peloquin 1984; Epperson et al. 2001; Marquardt et al. 2019). At higher elevations (2500–2650 m), which are cooler and more mesic, trees from the taxon previously described as Rocky Mountain ponderosa pine (*P. ponderosa* Lawson & C. Lawson var. *scopulorum* Engelm.) are the sole pines (Kearney and Peebles 1961; Kilgore, 2007). Although this taxon has predominantly three needles per fascicle (Conkle and Critchfield 1988), a recent study confirms that this taxon is not var. *scopulorum* Engelm., but rather more closely related to the Ponderosae of northern Mexico (Epperson et al. 2009; Willyard et al. 2017; Marquardt et al. 2019) and based on DNA and morphological characteristics was recognized as *P. ponderosa* var. *brachyptera* (Engelm.) Lemmon by Willyard et al. (2017). Herein, this taxon is referred as *P. ponderosa*. At lower elevations (1760–2400 m), which are warmer and more xeric, Arizona pine (*P. arizonica* Engelm.) is dominant; this taxon is closely related to *P. ponderosa* and has been classified by some as a subspecies (*P. ponderosa* var. *arizonica* (Engelm.) Shaw) (Kral 1993), although current classifications recognize it as a separate species (Price et al. 1998; Willyard et al. 2017) that is part of the ponderosa pine complex. *Pinus arizonica* has predominantly five needles per fascicle. At mid-elevations of this gradient (2430–2550 m), these taxa co-occur (Epperson et al. 2001; Kilgore 2007).

We examined the hydraulic traits of these two closely related taxa at both higher and lower elevation sites where they are mono-specific and within the mid-elevation transition zone where the two taxa co-occur. We predicted that hydraulic traits would differ between the mono-specific populations of the two taxa from the extremes of the elevational gradient, but that the co-occurring populations from the two *Pinus* taxa would not differ within the mid-elevation transition zone. This was predicted based on prior studies finding that some *Pinus* species vary in their hydraulic traits between populations and with differing growing conditions, leading to potential differences within a taxa between sites. For taxa at the site where they co-occur, some prior studies have found that *Pinus* species may be quite similar in some of their hydraulics traits when they are growing under the same environmental conditions (Oliveras et al. 2003; Gonzalez-Benecke et al. 2011). Trees growing at higher elevation were predicted to display hydraulic traits consistent with the more mesic environment than trees growing in more arid conditions at lower elevation. We measured embolism resistance (P_{50}), xylem specific hydraulic con-

ductivity (K_s), and seasonal leaf pressure potentials (Ψ_p).

METHODS

Study site and tree selection

The Santa Catalina Mountains rise from a basal elevation of 760 m to 2791 m at the highest point on Mount Lemmon in southern Arizona. Approximately 60% of the precipitation occurs during the summer monsoon season (July–September) with the remaining precipitation arriving during the winter months (Sheppard et al. 2002). The driest part of the year is the foresummer (May–June).

We investigated two closely related *Pinus* taxa within subsection Ponderosae at three sites across a moisture and elevational gradient. The high-elevation site contains only *P. ponderosa* and is near the summit of Mount Lemmon (32.4396°N 110.7871°W, 2770 m) with an interpolated (Wang et al. 2016) mean annual temperature (MAT) of 17.6°C and mean annual precipitation (MAP) of 569 mm for the period 1971–2000. The mid-elevation site contains both species and is located upslope from the Palisade Ranger Station (32.4138°N 110.7149°W, 2475 m), with a MAT of 18.4°C and MAP of 610 mm. The low-elevation sites contain only *P. arizonica* and are located near Lizard Rock (32.3844°N 110.6930°W, 2135 m) and Rose Canyon (32.3967°N 110.6932°W, 2165 m), with an average MAT of 19.1°C and MAP of 516 mm. In the years just prior to our measures (2001–2005), the high elevation site received 678 mm of annual precipitation (Mt. Lemmon ALERT Gauge, Pima County, AZ), and the mid-elevation site received 669 mm (White Tail ALERT Gauge, Pima County, AZ). For these years, we do not have climate data for the low elevation site, but it was markedly more arid than the other sites.

At each site, we identified trees by taxon and selected trees of similar size (mean tree height of 3.7 m and DBH of 12.6 cm) for measurements. Taxonomy was determined by their mean number of needles per fascicle across 5 yr of needles on at least one terminal branch because needle number separates these taxa, with *P. ponderosa* containing less than 3.2 needles per fascicle, while *P. arizonica* contains greater than 4.6 needles per fascicle (Peloquin 1984). We focused our sampling on trees with 3 needles per fascicle (*P. ponderosa* var. *brachyptera*) or five needles per fascicle (*P. arizonica*) to ensure that we were sampling individuals of known identity. Trees with intermediate or mixed needle number were not selected because they may represent hybrids (Peloquin 1984; Rehfeldt et al. 1996; Epperson et al. 2001, 2009).

Leaf Pressure Potentials

Leaf pressure potentials (Ψ_p) were measured from each of 3–8 trees per site and taxon in a single day

during the arid foresummer (10–20 June 2005), late summer monsoon wet season (6–12 August 2005), and winter (24–27 January 2006). From each tree, 5–10 current-year fascicles were collected from the terminal shoot on the second-lowest living branch (1.0–1.5 m above ground) on the south side of the tree at predawn and midday. Fascicles were cut from the tree using a fresh razor blade, placed in a plastic bag, and stored in a cooler with ice packs. Within 2.5 hrs of collection, Ψ_p from the fascicles were measured using a pressure chamber (PMS Instruments, Inc., Corvallis, OR). We measured several fascicles per tree to ensure that we were obtaining consistent values and then averaged samples by tree.

Stem Hydraulics

Stems were collected in the field on 20 and 26 October 2005 for determination of xylem specific hydraulic conductivity and vulnerability to cavitation. At predawn to early morning on each collection day, 5–11 trees per taxon per site were selected. From each tree, stems from the lowest living branch that were 5–10-mm diameter with minimal curvature were cut to 0.5 m in length, double-bagged in plastic bags with a moist paper towel, placed on ice, and transported via overnight shipping to Michigan State University. Stems were measured immediately following their arrival and within 3 d of collection.

In the laboratory, stems were trimmed under water from each end until a straight, unbranched segment 6–9 mm in diameter and 14 cm in length was obtained. Stems were then connected to a tubing system and flushed with low pH degassed water (pH 2 HCl; Sperry and Saliendra 1994; Sperry and Ikeda 1997; Pockman and Sperry 2000; Sperry et al. 1994) that had been passed through a 0.1- μ m filter. Stems were flushed for 1 hr at 30 kPa to remove gas emboli from stems (Hacke et al. 2000a; Hacke et al. 2007). This relatively low pressure was used in order to avoid aspiration of tori in pit membranes (Sperry et al. 2005). Conductivity increased with flushing. Following the initial one hour flush, hydraulic conductivity (K_h) of stems was measured, and stems were flushed for additional 20-min intervals until a constant maximum hydraulic conductivity (K_{hmax}) was obtained (usually less than 2 hr) (Hacke and Jansen 2009; Schoonmaker et al. 2010). Hydraulic conductivity of stems was measured gravimetrically (Sperry et al. 1988) using an analytical balance (Model BP 121 S, Sartorius AG, Goettingen, Germany) and a pressure head of <2 kPa. Conductivity was corrected for background flows (Hacke et al. 2000b). Xylem specific hydraulic conductivity (K_s) of stems was determined using the K_{hmax} and the cross-sectional xylem area. Cross-sectional xylem area (minus the pith) was determined for each stem. The K_{hmax} was then divided by this area for each stem to yield the xylem specific conductivity ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$). For K_s , 7–11 individuals were measured per site and taxon.

Following determination of K_s , a subset of stems (5–8 per species and site) were measured using a standard centrifuge technique (Alder et al. 1997) to generate vulnerability to cavitation curves. Stems were spun in a centrifuge (Sorvall RC-5B, DuPont Instruments, Wilmington, DE) using a custom-built rotor in order to generate known negative pressures. Stems were then reconnected to the tubing system, and the new hydraulic conductivity (K_s) was determined. This process was repeated with successive spins generating more negative pressures until stems experienced >80% loss in hydraulic conductivity. Vulnerability to cavitation curves were constructed by plotting the water potential (generated using the centrifuge) versus hydraulic conductivity, and the pressure at which 50% loss in conductivity occurred for each sample (P_{50}) was obtained for each sample using a Weibull curve (Microsoft Excel 2010, Microsoft, Redmond, WA). When comparing curves across the taxa and sites, curves were plotted as the water potential versus K_s because several studies have now highlighted the importance of consideration of K_s when comparing across curves and the potential difficulty in interpreting and evaluating curves that show only relative losses (Sperry et al. 2012; Jacobsen and Pratt 2012; Hacke et al. 2015).

Data Analyses

The elevational range for each taxon overlapped, with the ends of the ranges occupied by only one taxon. Because of the nature of these distribution limits for each species, with both species not occurring across all three sites, the effects of site (i.e., comparing across the low, mid, and high elevation sites) could not be examined across the entire study nor could an interaction between site and taxon be evaluated. This limitation was part of the original study design and did not impact the comparisons that we were most interested in, which were pre-planned contrasts of hydraulic trait variation between the two taxa from their mono-specific populations and the two taxa at the site where they co-occurred. Consequently, our analyses included each taxon \times site as a separate treatment (i.e., we ran an ANOVA comparing data from four groups: *P. ponderosa* at the upper site, *P. ponderosa* at the mid site, *P. arizonica* at the mid site, and *P. arizonica* at the lower site). This approach allowed us to calculate the correct error term across the analysis, while analyzing the contrasts that were the focus of the present study. This analysis precluded a comparison of the two taxa pooled across the sites at which they occurred, but this comparison was not important for addressing our experimental question. We used an alpha of 0.5 for our ANOVA and conducted comparisons among groups using Tukey Pairwise comparisons. All analyses were run using Minitab 17 (v. 17.2.1, Minitab, Inc., State College, PA, USA).

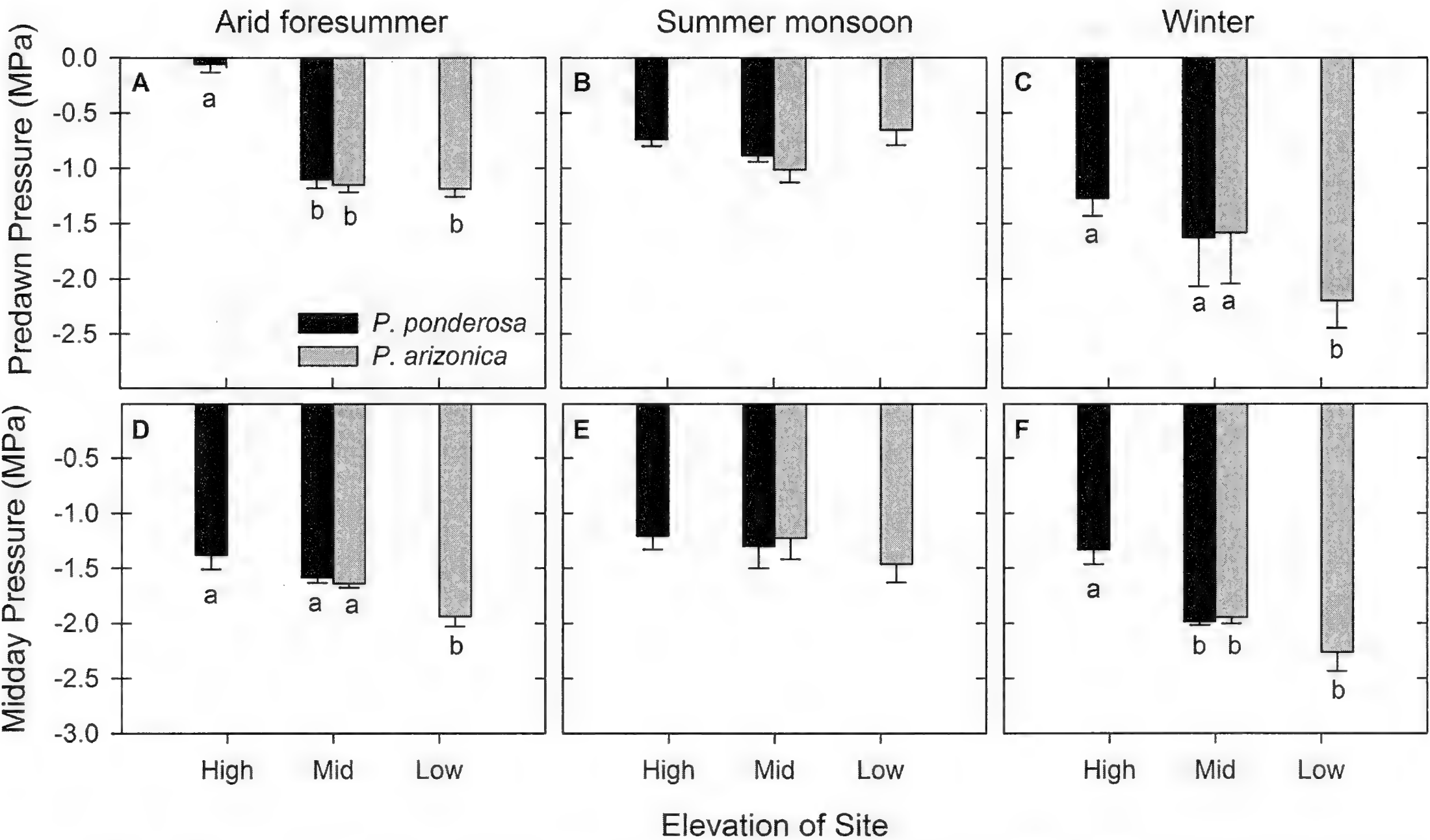


FIG. 1. The seasonal mean (± 1 SE) leaf pressure potentials at predawn (A–C) and midday (D–F) for two *Pinus* taxa, *P. ponderosa* and *P. arizonica*, from sites at three different elevations (High, Mid, and Low) across three seasons (Arid foresummer, Summer monsoon, and Winter). For panels A, C, D, and F, bars with different lowercase letters are significantly different. There were no significant differences among taxa and sites for the data shown in panels B and E.

RESULTS

Leaf Pressure Potentials

Trees from higher elevation had significantly higher Ψ_p than trees from lower elevations at predawn and midday during the arid foresummer (Fig. 1A) and winter (Fig. 1F). The trees at the lowest elevation had significantly more negative Ψ_p at midday during the arid foresummer (Fig. 1D) and predawn during the winter (Fig. 1C). Where they co-occurred at the mid-elevation site, Ψ_p were not different between the taxa during any of the sampled periods (Fig. 1; Table 1).

Pressure potentials were most negative for both taxa in the winter and least negative during the monsoon season, with the arid foresummer intermediate. There were no differences between taxa or sites during the moist monsoon season (Fig. 1B, E; $F_{3,18} = 2.01$, $P = 0.149$, predawn; $F_{3,18} = 0.18$, $P = 0.907$, midday). However, there were differences among sites and taxa during the arid foresummer (Fig. 1A, D; $F_{3,18} = 8.28$, $P = 0.001$, predawn; $F_{3,18} = 8.32$, $P = 0.001$, midday) and winter (Fig. 1C, F; $F_{3,18} = 11.11$, $P < 0.001$, predawn; $F_{3,16} = 15.64$, $P < 0.001$, midday).

TABLE 1. PREDAWN AND MIDDAY LEAF PRESSURE POTENTIALS (MPa; MEAN \pm 1 SE) FOR TWO *PINUS* TAXA, *P. PONDEROSA* AND *P. ARIZONICA*, FROM SITES AT THREE DIFFERENT ELEVATIONS (HIGH, MID, AND LOW) ACROSS THREE SEASONS (ARID FORESUMMER, SUMMER MONSOON, AND WINTER). Each taxon only occurred across two of the sampled elevations, and N/A is reported if a taxon did not occur at a given site. Significant differences between sites and species within a season are shown in Fig. 1.

Site	Arid foresummer		Summer monsoon		Winter	
	<i>P. ponderosa</i>	<i>P. arizonica</i>	<i>P. ponderosa</i>	<i>P. arizonica</i>	<i>P. ponderosa</i>	<i>P. arizonica</i>
Predawn						
High	-0.06 ± 0.08	N/A	-0.74 ± 0.06	N/A	-1.38 ± 0.13	N/A
Mid	-1.10 ± 0.08	-1.15 ± 0.07	-0.88 ± 0.06	-1.01 ± 0.11	-1.59 ± 0.05	-1.64 ± 0.04
Low	N/A	-1.19 ± 0.07	N/A	-0.65 ± 0.14	N/A	-1.94 ± 0.09
Midday						
High	-1.27 ± 0.16	N/A	-1.21 ± 0.12	N/A	-1.33 ± 0.13	N/A
Mid	-1.63 ± 0.44	-1.58 ± 0.47	-1.30 ± 0.20	-1.23 ± 0.19	-1.98 ± 0.03	-1.95 ± 0.06
Low	N/A	-2.20 ± 0.25	N/A	-1.46 ± 0.17	N/A	-2.26 ± 0.17

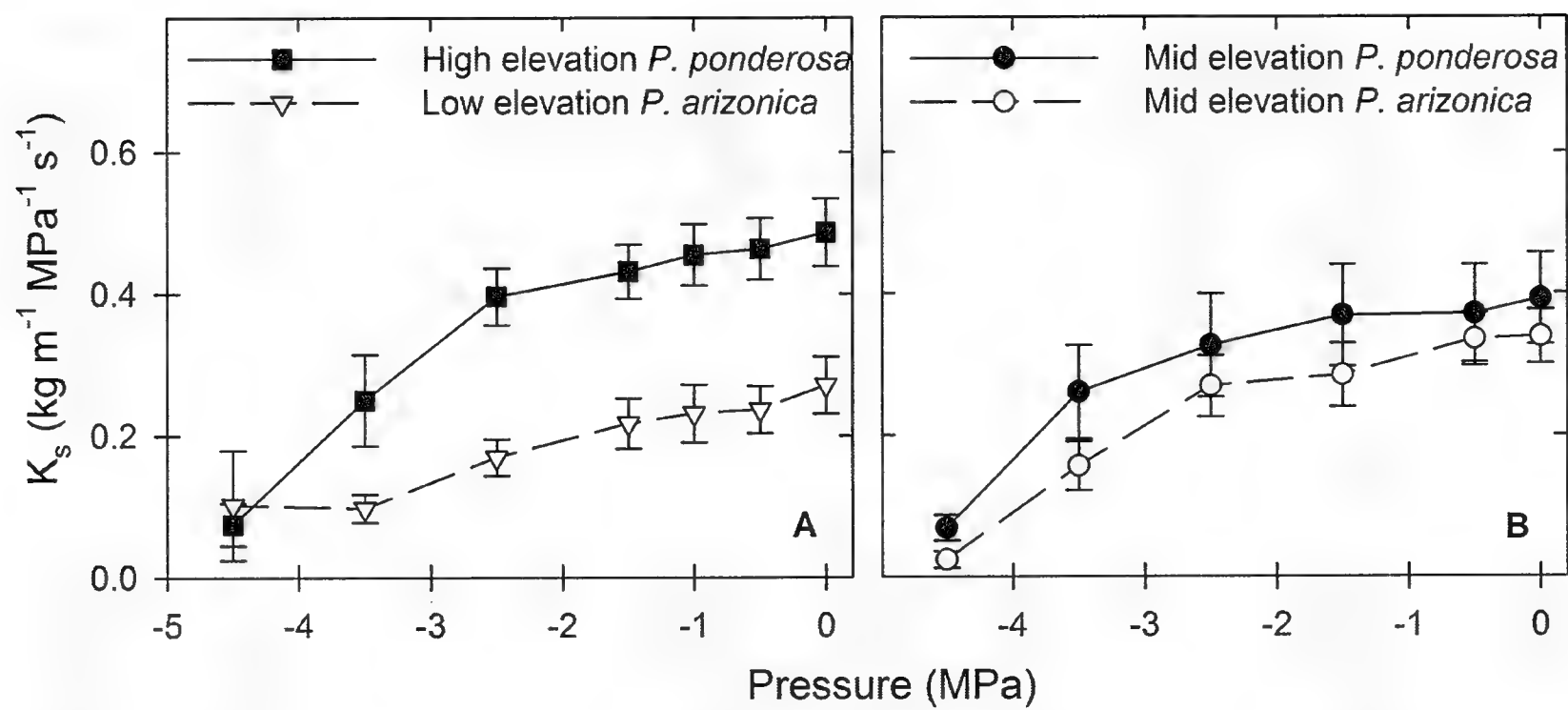


FIG. 2. Vulnerability to cavitation curves for two *Pinus* taxa, *P. ponderosa* and *P. arizonica*, with the decline in mean (± 1 SE) xylem specific conductivity (K_s) with pressure for three different sites: (A) a high elevation site containing only *P. ponderosa* compared to a low elevation site containing only *P. arizonica*, and (B) a mid-elevation site where both taxa co-occur.

Stem Hydraulics

Vulnerability to cavitation curves varied for the two taxa depending on site (Fig. 2; Table 2). When the two taxa were compared from the two sites where each occurred mono-specifically (i.e., *P. ponderosa* at the upper site compared to *P. arizonica* at the lower site), the curves of the two taxa varied greatly (Fig. 2A). In contrast, when the curves for these two taxa were plotted from individuals measured from the mid elevation site where they both co-occurred, there was no difference between the curves of the two species (Fig. 2B).

K_s varied significantly with taxon by site (Fig. 3A; Table 2; $F_{3,30} = 4.75$, $P = 0.008$). Consistent with the pattern evident from the vulnerability to cavitation curves, K_s was significantly different when the two taxa were compared from the high and low elevation sites where they each occurred mono-specifically, but they were not different from one another at the mid elevation site where they co-occurred. P_{50} did not vary with taxon by site (Fig. 3B; $F_{3,23} = 1.03$, $P = 0.399$). This may have been due to the smaller sample size for this comparison because K_s and P_{50} were strongly correlated suggesting that changes in K_s should have corresponded to changes in P_{50} . K_s was predictive of P_{50} for *P. ponderosa* (Fig. 4; $F_{13} = 5.70$, $P = 0.034$, $r^2 = 0.32$) and for all of the data pooled across all sites and taxa ($F_{23} = 10.90$, $P = 0.003$, $r^2 = 0.33$), but not

for the *P. arizonica* analyzed alone ($F_9 = 1.32$, $P = 0.285$, $r^2 = 0.14$). K_s was negatively correlated with P_{50} , such that increased K_s corresponded to a decreased (i.e., more negative) P_{50} .

DISCUSSION

We found that the comparative hydraulic traits of two closely related *Pinus* taxa differed depending on where they were sampled. When they were compared using data gathered from trees occurring at a high elevation and low elevation site where the taxa do not co-occur, the taxa significantly differed in their hydraulic conductivity. When these two taxa were compared at a mid-elevation site where they co-occurred, the taxa were not different in their hydraulic conductivity. This variation in traits within taxa from populations experiencing different climatic conditions and occurring at different sites is consistent with several studies on both gymnosperm (Tognetti et al. 1997; Maherali and DeLucia 2000; Martínez-Vilalta et al. 2009) and angiosperm (Mencuccini and Comstock 1997; Kavanagh et al. 1999; Kolb and Sperry 1999; Pratt et al. 2012; Jacobsen et al. 2014) species. However, this variability may be species or population specific, and several studies have found that hydraulic traits did not vary among studied populations (Mencuccini and Comstock

TABLE 2. MEAN (± 1 SE) XYLEM SPECIFIC CONDUCTIVITY (K_s) AND PRESSURE AT 50% LOSS IN HYDRAULIC CONDUCTIVITY (P_{50}) FOR TWO *PINUS* TAXA, *P. PONDEROSA* AND *P. ARIZONICA*, FROM SITES AT THREE DIFFERENT ELEVATIONS (HIGH, MID, AND LOW). Each taxon only occurred across two of the sampled elevations, and N/A is reported if a taxon did not occur at a given site. Significant differences between sites and species within a season are shown in Fig. 3.

Site	K_s (kg m ⁻¹ MPa ⁻¹ s ⁻¹)		P_{50} (MPa)	
	<i>P. ponderosa</i>	<i>P. arizonica</i>	<i>P. ponderosa</i>	<i>P. arizonica</i>
High	0.48 \pm 0.03	N/A	-3.43 \pm 0.23	N/A
Mid	0.36 \pm 0.07	0.34 \pm 0.04	-3.36 \pm 0.18	-3.15 \pm 0.21
Low	N/A	0.26 \pm 0.04	N/A	-2.95 \pm 0.13

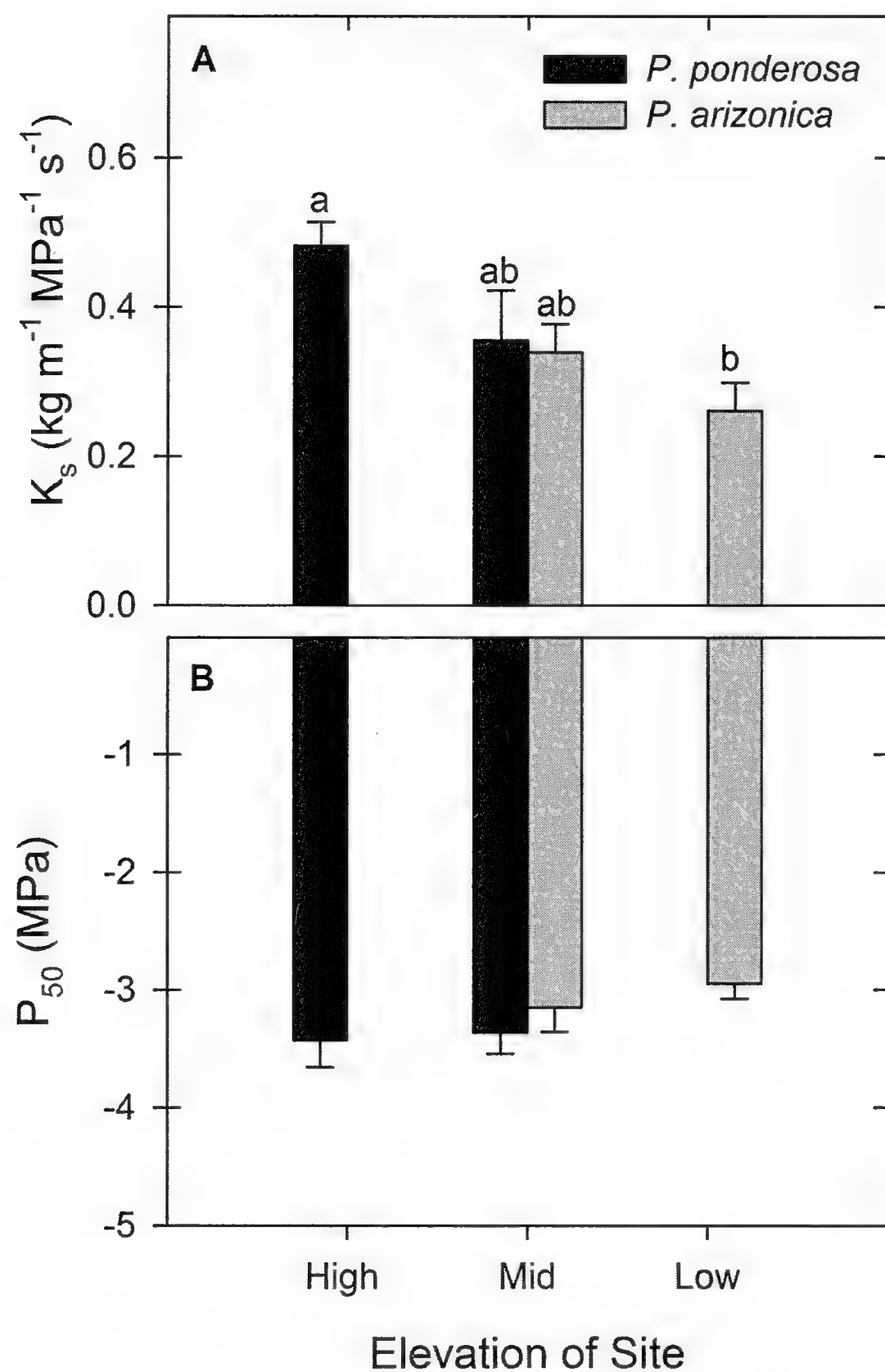


FIG. 3. The mean (± 1 SE) (A) xylem specific conductivity (K_s) and (B) pressure at 50% loss in hydraulic conductivity (P_{50}) for two *Pinus* taxa, *P. ponderosa* and *P. arizonica*, from across an elevation gradient. For panel A, bars with different lowercase letters are significantly different. There were no significant differences among taxa and sites for the data shown in panel B.

1997; Matzner et al. 2001; Martínez-Vilalta and Piñol 2002; Stout and Sala 2003; Lamy et al. 2011).

The different hydraulic traits occurring across the studied elevation transect may be due to either plasticity in xylem structure and functional or genetic variation among populations. In *P. ponderosa*, a previous study found differences in hydraulic conductivity among desert and montane populations, but these differences were not maintained in a common garden, suggesting that they were due to xylem plasticity (Maherali and DeLucia 2000). For another species, *P. halepensis*, hydraulic trait differences were found in common garden experiments, suggesting that there was genetic variation in hydraulic traits among populations (Tognetti et al. 1997; David-Schwartz et al. 2016). Common garden and controlled experiments will likely be instrumental in future studies examining the ability of plants to respond to varying conditions and the impact of plasticity versus genetics in confirming field-based findings of variation (Holste et al. 2006; Beikircher

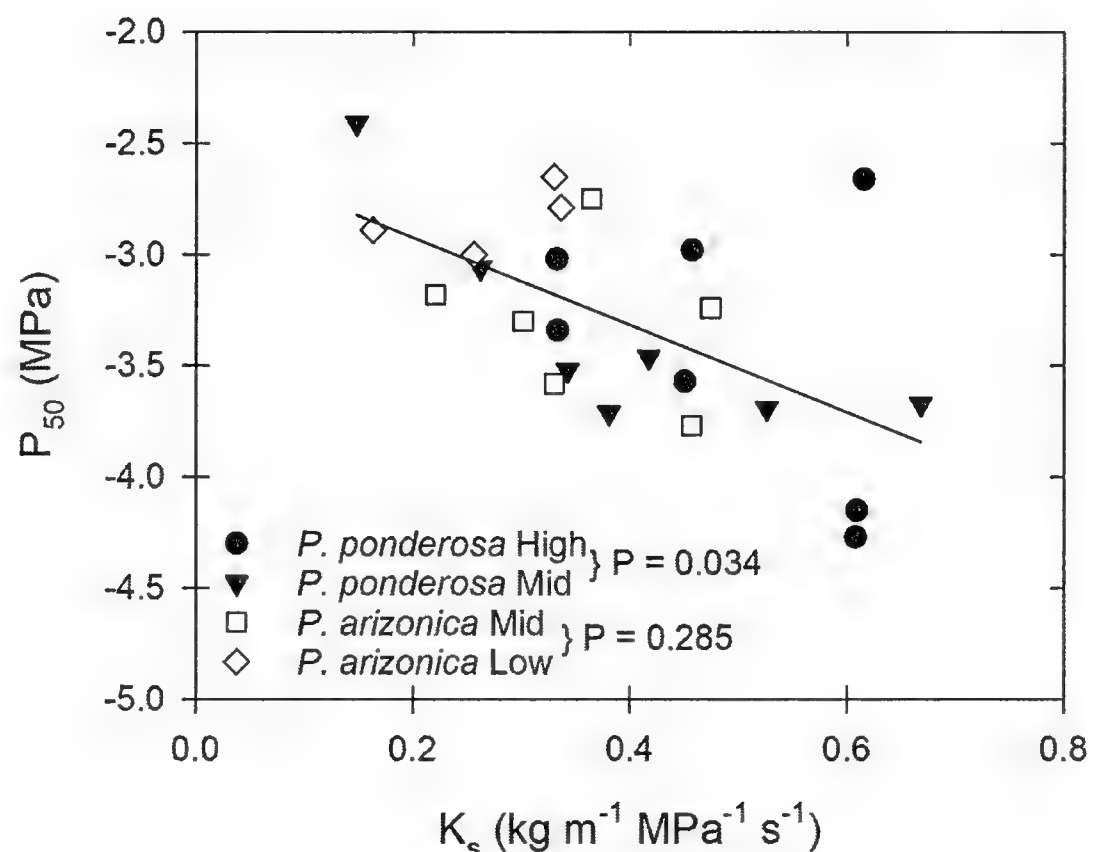


FIG. 4. Xylem specific conductivity (K_s) as a predictor for the pressure at 50% loss in hydraulic conductivity (P_{50}) for two *Pinus* taxa, *P. ponderosa* (filled circles) and *P. arizonica* (open circles). K_s was predictive of the pressure at 50PLC for *P. ponderosa* (solid line) but not for *P. arizonica*.

and Mayr 2009; Mayr et al. 2010; Fichot et al. 2010; Awad et al. 2010; Plavcová and Hacke 2012).

In the present study, both taxa exhibited similar hydraulic traits at the site where they co-occurred. This suggests that there may be a common “solution” for *Pinus* for persistence within the conditions of that specific site. Both taxa were able to occur there because they were each able to achieve this specific functional trait value. The transition zone may, therefore, be delimited by limits on the ability of each taxon to further respond to changing climate conditions, which prevented the *P. ponderosa* from moving into sites any farther downslope or the *P. arizonica* from moving upslope. Their co-occurrence also suggests that competition between the two may be limited and that physiological tolerance traits are key in determining distributions in this system.

Hydraulic tolerances of *Pinus* species may be particularly important due to their apparent sensitivity to drought and water stress. The K_s and P_{50} for the taxa included in this study are similar to those previously reported for *Pinus ponderosa* (Maherali and DeLucia 2000; Stout and Sala 2003). Several studies have described recent mortality events of *Pinus* that have been associated with water stress (Allen and Breshears 1998; Bigler et al. 2006; McDowell et al. 2009). In the present study, water potentials were relatively high compared to the cavitation resistance of plants, although our study did not occur during a particularly dry year. However, plants may have very different tolerances of embolism. For instance, some desert plants appear to regularly reach very high levels of embolism without experiencing dieback or mortality (Jacobsen et al. 2007; de Dios Miranda et al. 2010), while other species may be sensitive to lower levels of embolism (~60%) (Sperry and Love 2015).

Absolute levels of conductivity (Sperry et al. 2012; Jacobsen and Pratt 2012; Hacke et al. 2015) are also important to consider, and pines, which have relatively low maximum hydraulic conductivity, may be sensitive to even small declines in supply. The ability to tolerate very low flows, even for brief periods of time, varies among species (Pratt et al. 2005) and links leaf-level traits associated with minimizing water loss to hydraulic supply traits. Compared to *P. ponderosa*, *P. arizonica* is better able to maintain photosynthetic function and limit water loss from needles when dehydrated (Kilgore 2007), which may explain the ability of this species to persist in drier sites or to tolerate the higher levels of embolism and lower hydraulic conductance than it experiences as a result of being both more vulnerable and having lower conductivity.

Another way of examining the hydraulic limits of species is through the hydraulic safety margin, which is the difference between the minimum water potential experienced and vulnerability to embolism (Oliveras et al. 2003; Meinzer et al. 2009; Ramirez et al. 2020). In a recent study comparing the hydraulics of populations, the hydraulic safety margin was useful in identifying intra-specific populations that were hydraulically buffered compared to those that were not (Ramirez et al. 2020). In the present study, high elevation *P. ponderosa* showed the highest hydraulic safety margin and low elevation *P. arizonica* showed the lowest safety margin. For both taxa, the lowest hydraulic safety margins, and most at-risk populations, were those occurring at the lower elevation site for each. This suggests that lower elevation distributions of these species may be most at risk of drought-associated mortality.

In summary, we found evidence of hydraulic trait differentiation between two closely related taxa and hydraulic trait differentiation among populations growing at different elevations. This is an important finding in our developing knowledge of intra-specific variability in plant hydraulic function. The presence of population variability in this and other studies suggests that plant hydraulic studies should more carefully consider these sub-specific patterns in experimental designs and analyses. This may be particularly important in the context of conservation planning and climate change studies because variable versus homogenous species tolerances can greatly alter model outputs (Valladares et al. 2014). This may also be important to consider when examining drought-associated patterns of mortality as linked to functional traits because drought events often affect ecotones and populations at species range margins most strongly (Allen and Breshears 1998; Paddock et al. 2013), and species traits from other populations may not well represent the traits of these susceptible populations.

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CBS PRESIDENT'S REPORT FOR VOLUME 67

Dear Colleagues,

Normally, this end-of-year President's Report would have been written from start to finish by the recently elected incoming President of the Society: a botanist who, normally, would have been elected by affirmation among wine-fueled toasts at the Society's annual banquet, which, normally, would have been held in the previous spring. This new President would, normally, have had the benefit of several months' worth of Council meetings to become familiar with the current year's milestones, activities, and discussions before embarking on the, normally, happy task of writing their first President's Report. In any normal year, the first two sentences of President's Report would not include five uses of the words "normally." But the year 2020 has been so rife with exceptions, delays, shattered protocols, professional catastrophes, and personal tragedies that few formalities — including the drafting of this report — have remained untouched. Below, we reflect on the ways in which the COVID-19 pandemic affected the ebb and flow of Society business as well as on the ways in which, by forging ahead to conduct business as usual, we enjoyed some very bright spots, too.

The pandemic's first mark on our year took form in the Council's decision to postpone the 2020 annual banquet – scheduled to take place in early April – for what we first thought might be only a few months, but what we now know will be for more than a year. Given all that has happened since April 2020, it's hard to imagine now how difficult this decision was when we had to make the call in early March. Were we being too cautious? Had we lost our sense of adventure? Would it be safe for the Society president (in lockdown in New Haven, CT) to fly to San Francisco? None of us has any second thoughts now about the wisdom of the judgement to cancel the banquet, but it was a decision not lightly taken, as the banquet is such a high point of our year. Moreover, Brett Hall, our host for the event, which was to take place in the Arboretum of the University of California, Santa Cruz, had planned a set of festivities that the Council was anticipating with great joy.

The pandemic's second mark on the Society's annual cycle was its effect on the transition between presidents. Between stay-at-home orders, cramped home offices, remote teaching, recording lectures, and the childcare challenges that boggled millions of minds throughout the western U.S., the pandemic disrupted everyone's lives so deeply that the Council realized that we had to re-think how best to approach the usually exciting and high-spirited challenge of selecting and successfully inviting a (normally)

unsuspecting colleague to serve a three-year presidential term. We recognized, too, that the pandemic had unambiguously reduced to a tiny sliver everyone's "extra" bandwidth (which is never abundant to begin with!) for taking on new responsibilities. So, we wanted to wait a bit until everyone had time to adjust to the "new normal" before seeking a new president. And, without a banquet packed with post-field-trip botanists ready to pass the proverbial torch, the Council had to devise a Plan B for how to transfer the office of president. So, in short, the Council was slow to pop the question to the candidates who we coveted for the job, and it took a little longer than usual for the decision to be made. Happily, in a great stroke of luck, the Society's incoming President, Dr. Amy Litt, accepted the Council's invitation to lead the Society for the next three years, while the outgoing President, Dr. Susan Mazer, promised to show her the ropes. Dr. Litt's election was cemented by the Society's members through an online vote, but the protracted process resulted in yet another mark of the pandemic on the Society, which is that this President's Report is being written not by one president, but by both of us. In short, this is not a President's Report, but a Presidents' Report.

As the two of us considered the past year, we noted several bright glimmers that are worthy of note. First is the Council itself. The Society has greatly benefitted from the insights and suggestions of its First Vice President, Dr. Joshua Der (California State University, Fullerton), which are consistently innovative and productive. Similarly, the Council's two new members at large, Dr. Muriel Poston (Pitzer College) and Dr. Nishi Rajakaruna (Cal Poly, San Luis Obispo), and our Outreach Coordinator, Dr. Lorena Villanueva-Almanza (a recent PhD from the University of California, Riverside), provide both energy and wisdom that will help us to design and to usher in our collective commitment to increase diversity in, and accessibility of, the botanical professions. Our newsletter, *Nemophila*, was designed and published under the creative hand of Josie Lesage (PhD student, University of California, Santa Cruz), a position that in the upcoming year will be assumed by Dr. Rachael Olliff Yang (a newly minted PhD at the University of California, Berkeley), who will be stepping down as our Membership Chair. And, of great importance, looking back through the whole of the last presidential term, Susan Mazer is particularly grateful for the guidance and steel-trap-historical-memories of Nancy Morin (Recording Secretary), David Margolies (Treasurer), Andrew Doran (Member at Large), and the tireless leadership of *Madroño* Editor in Chief, Dr. Justen Whittall (Santa Clara University),

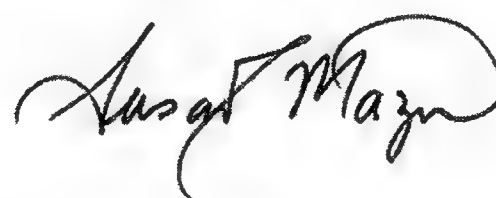
who also took on double duty in his service as a judge and award-granter at the Santa Cruz and Santa Clara County (virtual) science fairs in spring 2020. The four \$100 first-place awards and two \$50 second-place awards granted to these young botanists by the Society are perhaps the happiest payments we made this year, and were likely among the most appreciated checks received!

But the science fair award winners weren't the only promising botanists to receive funds from the Society in 2020. In spring, we received 13 compelling applications for the Paul Silva Student Grants, awarded annually. As was the case in 2019, the Council was unable to restrain ourselves from granting four awards (up from the historical average of two awards per year). Ernesto Chavez-Velasco (University of California, Santa Cruz) will use his grant to investigate interactions between coastal fog and drought, and how they affect water use and endophytic fungi in the coastal prairies of California. We've all wondered whether and how coastal fog may mitigate drought stress, but Ernesto's work will take the extra steps of examining how fog may affect fungal virulence and how moisture may affect the ability of endophytic fungi to become established. Jenna Ekwealor (University of California, Berkeley) will explore the roles and genetic basis of UV tolerance and UV protection in mosses of the Mojave Desert. How do those gametophytes do it? Jacob Ewald (California State University, Chico) will conduct a challenging project on *Mimulus guttatus* and *M. glaucescens* to evaluate which of 14 prospective reproductive isolating mechanisms prevent hybridization between these species in the wild (or *do* they hybridize after all?). Finally, Michael Mulrow (California State University, Chico) will examine the factors determining community composition in lichen communities of ultramafic and sandstone outcrops across a maritime gradient in California. The combinations of field, greenhouse, and lab work represented by these studies hold the promise of unraveling the kinds of vexing botanical mysteries that are impossible to ignore if you spend any time observing plants outdoors.

Several other hopeful thoughts and plans for action comprise our consideration of 2020. Now that we're all so Zoom-adapted, the feasibility of reaching large audiences to spread the word about western U.S. botanical research has increased exponentially. Accordingly, the Council is now consider-

ing how to take advantage of this medium to promote short-talks, mini-symposia, dissertation defenses, and full research seminars of Society students, postdocs, and faculty, particularly botanists of color. We know that educators are increasingly recognizing, and helping students to appreciate, the contributions and accomplishments of botanists of color (explore the *Growing Black Roots: The Black Botanical Legacy* lecture series sponsored by Holden Forests & Gardens), and the Council looks forward to assembling and disseminating among educators ways in which we can all make our courses and research groups maximally welcoming to everyone who has a soft spot for plants. The future of Botany – and the natural world – depends on such openness, and we welcome your ideas for how we can achieve it. And, while we're on the topic, please don't hesitate to contact us or anyone else on the Council if you would like to consider serving as a Council member or to suggest a prospective member for our consideration. We're currently seeking a new Membership Chair, Corresponding Secretary, and Outreach Coordinator, so if you're looking for one of the most engaging service positions available anywhere, this could be a great match.

Sincerely,



Susan J. Mazer
Past President (2017-2020)
Professor of Ecology and Evolution
University of California, Santa Barbara
Email: sjmazer@ucsb.edu



Amy Litt
Current President (2020-2024)
Professor of Botany
University of California, Riverside
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EDITOR'S REPORT FOR VOLUME 67

I am pleased to report the publication of *Madroño* volume 67 by the California Botanical Society for 2020.

In my third year editing *Madroño*, I have attempted to keep *Madroño* at the leading edge of peer reviewed, scientific publications in natural history. Highlights from this past year include publishing 11 full-length Contents articles (nearly 2x more than last year), five Noteworthy Collections, three Book Reviews, and two manuscripts describing New Species. Issue 3 was dedicated to a colorful and thorough Checklist of the Vascular Plants of Mulegé, Baja California Sur, Mexico (Valov).

We continue to include Open Access articles to the *Madroño* library including one in this very issue (Coltharp et al.). Our contributions from graduate students and undergraduate coauthors continue to grow thanks to outreach efforts from the California Botanical Society Board and our membership.

We continue to publish numerous color plates in each issue. We are grateful for the efforts of 32 distinct reviewers, several of which reviewed multiple contributions this past year. The list of reviewers found in this issue also includes numerous graduate students participating in our reviewer-in-training program through California Botanical Society's outreach program (a.k.a. The Botany Ambassadors).

The average time from submission to publication for *Madroño* hovers between six and eight months with occasional delays due to COVID. We believe that *Madroño* is still the best outlet for western botanists to publish their discoveries in a timely fashion, while reaching a dedicated and passionate audience. We are always on the lookout for Special

Issue topics and Guest Editors. We are currently accepting proposals for Special Issues that highlight any aspect of western botany at any scale of biological organization (from molecules to ecosystems). If you are interested in proposing a special issue or would like to discuss your ideas for a special issue, please contact me (madronoeditor@gmail.com). Spoiler, we have two Special Issues coming in 2021 – one containing a series of bryophyte checklists and floras from western North America and the other focused on phenological patterns in western North American plants.

The efforts of numerous individuals are critical to the successful publication of *Madroño* including a team of Associate Editors and a pair of amazing administrative assistants. Our team of Associate Editors includes Book Editor Matt Ritter, Noteworthy Collections Editor David J. Keil, Copy Editor Alisa Hove, and our Index/TOC Editor Steve Timbrook. Administrative support is provided by Lynn Yamashita and Rachael Olliff-Yang.

I find great pleasure in helping cultivate manuscripts from the submission process through to the printed copy that arrives in our mailboxes. Being on the forefront of discoveries in western botany and working with such astute and passionate authors and reviewers is a pleasure. Botany is alive and well in western North America! The evidence is in *Madroño*. Please continue to submit your work to the journal, recruit new authors, share links to the papers often (rather than posting the PDFs) and have a great year.

Justen Whittall

December 2020

REVIEWERS OF MADROÑO MANUSCRIPTS 2020

Aaron Ramirez
Alisa Hove
Beth Roskilly
C. Matt Guilliams
David J. Keil
Dean W. Taylor
Debra R. Ayres
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Matt Ritter
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Nancy Morin
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Nishanta Rajakaruna
Rachael Olliff-Yang
Rick Karban
Robert Preston
Sierra Lauman
Sula Vanderplank.
Susan Lambrecht
Tasha LeDoux
Tom Daniel

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